STUDIES ON THE PATHOLOGICAL CHANGES IN EXPERIMENTAL FLUORINE POISONING IN SHEEP

Thesis

Submitted to the Faculty of Veterinary Science,

RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR

in partial fulfilment of the requirements

for the degree of

MASTER OF SCIENCE (VETERINARY)

IN

PATHOLOGY

By

Sidh Nath Tiwary,

B. V. Sc & A. H. (R.A.U.),

JUNIOR RESEARCH FELLOW (R.A.U.), BIHAR

DEPARTMENT OF PATHOLOGY,

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Dated, the 6th June 1975.

This is to certify that the work embodied in this Thesis entitled "STUDIES ON PATHOLOGICAL CHANGES IN EXPERIMENTAL FLUORINE POISONING IN SHEEP", submitted for the award of Master Degree of Science (Veterinary Pathology) of Rajendra Agricultural University, Bihar is the bonafide research work of Sri Sidh Nath Tiwary and was carried out under my guidance and supervision, and that incorporates the results of his independent study.

(C. D. N. SINGH)

CERTIFICATE

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

Tiwary.

(Sidh Nath Tiwary)

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(Sidh Nath Tiwary)

DEDICATED

TO

MY

PARENTS

CONTENTS

INTRODUCTION

INTRODUCTION

"A HEALTHY MIND DWELLS IN A HEALTHY BODY"

But all this depends upon availability of right type of food. Food of animal origin has a higher nutritional value and provides certain required nutrients which cannot be adequately obtained from many plants.

In the Rigveda there are hymns and there are references to the bleaching and spinning of wool. A verse from Subhashitani is quoted below:

कार्यपिदाो जनः प्रायः प्रीतिमा विष्करोत्यलम् ।
लोभार्थी शौण्डिकः शब्यैर्नंग पुष्णाति पेशलैः ।।

(A man loves more to others when in need. As Shephered in greed of wool serves more to their sheep and get them fattened with green grasses).

In India there are 40.22 million sheep producing 33,298 metric tonnes of wool, valued at rupees 122.6 million per annum. In 1959-60, 15.3 million sheep skins valued at 68.3 million rupees were produced.

With all its qualities of wonderful farm animal, the condition of sheep industry in India is still deplorable. The

productive capacity of the sheep in India is very low as compared with other foreign countries. Now in India people have realised the importance of sheep. The Indian Council of Agricultural Research has formulated ambitious plans and research scheme for the development of sheep.

Sheep suffer from several diseases and many of them are known to die from parasitic, viral and bacterial infection at times. Minett (1949) has conducted a survey on mortality of sheep and goat in India and he came to the conclusion that helminthic disease was an outstanding source of mortality.

Deficiency diseases and different poisonings cause deaths of sheep. Fluorine poisoning has hither to attract notices of several workers in India and abroad.

Fluorine is present in most plant tissues because of its wide spread occurrence in the soil, water and rock. A greater concentration is found in legume plants than in grasses. Fluorine is normally a constituent of animal tissues, particularly in herbivorous animals which feed upon these plants directly.

Fluorine toxicity in domestic animals may be acute or chronic. The toxic effect of fluorine is related to the solubility and absorbability of the fluorides, the quality of feeds ingested, duration of ingestion, degree of storage in the bones and teeth

and presence or absence of physiological stress, e.g. growth, lactation, pregnancy, inadequate nutrition, disease and climatic condition.

The signs of acute fluorine poisoning are an immediate loss of appetite, progressive emaciation, and debilitation leading to death. Chronic fluorine poisoning results from the prolonged and excessive ingestion of fluorine in mineral supplements, drinking water, feeds, concentrates and contaminated forages.

Fluorine poisoning of human beings and farm animals has formed the subject of study in our country. Clinical observations have not always proved very helpful, because of the well known fact that osteomalacia and chronic fluorosis in ruminants can not be differenciated by purely clinical methods, as the syndrome in both the diseases is very similar.

Young calves showing brown pigmentation were found in Madras Presidency. These were diagnosed to be a case of fluorosis. Fluorine content in water in the affected areas is as high as 2.5 parts per million. Nalanda and Nawada are suspected zones of fluorosis in Bihar. West Godavari, Nalgonda, Nellore and Anantpur are reported to be suspected areas of fluorosis in Andhra Pradesh.

All these places are situated in the vicinity of mountain ranges. Many of these places are near the sites of old volcanic regions. Hot or sulphur springs are still in existence in the vicinity. It is thus possible, as suggested by the Wilson (1939) that in all these regions there is substratum of rocks which includes lavas and associated granites and cryolites giving rise to an increased fluorine content of the soil and water.

Detail study on the fluorine poisoning may unfold the different fact of this emergent disease prevailing in our State.

The object of this research work is to throw light on the pathologic changes in sheep poisoned experimentally with fluorine in order to understand the pathological changes in sheep and also correlates these changes with those occurring in natural cases of fluorine poisoning. The findings on the pathological changes will be also profitable to future research worker on this disease in sheep.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

Moissan (1886) was the first man to isolate fluorine.

As an element, it is a pungent, poisonous, and yellow-green gas. It is a dangerous material and one of the most reactive elements known so far. It does not remain as an element in nature but always exists in some compound form.

Some of the important compounds of fluorine which are found in nature are Aluminium trifluoride, Cryolite, Boron trifluoride, Fluoboric acid, Hydrogen fluoride, Fluosilicic acid, Polytetra fluoroethylene, Tetra fluoroethylene, Polychlorotrifluoroethylene, Polyvinyle fluoride, Sodium fluoride, Calcium fluoride, Apatite and Rock phosphate etc.

Fluorine is frequently found in water from underground sources, particularly in areas where rock phosphate is found. It is also present in most plant tissues because of its wide spread occurrence in the soil, water and rock.

Lilleengen (1934) described the histological examination of the bones of sheep suffering from chronic fluorine poisoning. The bones examined originated chiefly from experimentally produced chronic fluorosis. The changes have a strong resemblance to those found in typical osteomalacia.

Roholum (1934) reported fluorosis in Iceland after volcanic eruption. The sight of some bones from Iceland sheep, which had died after volcanic eruption caused the author to investigate the condition, as the bones, showed similar changes to those of animal poisoned by fluorine.

Velu (1934) described that under experimental condition in the case of chronic fluorine poisoning, it required at least six months to produce teeth lesions in sheep.

Henry and Benzamin (1936) analysed the fluorine content of some phosphatic materials used as mineral supplements in the feeding of sheep in Southwales. The results showed that fluorine is present in almost negligible amounts in samples of dicalcic phosphate (0.127%), bone meal (0.068%) and a bone meal product (0.33%). Ground rock phosphate contained as much as 2.511% and a superphosphate 1.360%. It is pointed out that if sheep consumed 3.5 oz. of a lick composed of four parts of salt and one part of rock phosphate, they would ingest 80 mg of fluorine per day which has been shown to interfere with the development of the teeth after two years.

According to Roholm (1937), fluorine occurs in nature in rocks and minerals such as apatite, cryolite, fluorspar and phosphorite and traces of it are found in certain soils and

and water. Small quantities were found in plant and animal tissues specially in bone ash (0.01 to 0.04%). He described the signs of acute fluorine poisoning. Acute poisoning was manifested by local irritation or corrosion and by symptoms due to absorption. Haemorrhagic vomiting, diffuse abdominal pains, weakness and diarrhoea accompanied by a lowering of blood pressure were among the usual symptoms. Postmortem examination revealed haemorrhagic gastroenteritis, acute nephritis and varying parenchymatous degeneration of the viscera. Lethal dose of sodium fluoride for lower mammals was 23 to 90 mg. per kg. body weight. Chronic poisoning of fluorine principally caused degenerative tooth changes, diffuse osteomalacia and a generalised osteomalacia like bone diseases.

Peirce (1938) made observations on the toxicity of fluorine in sheep. Phosphatic licks, bone meal and dicalcic phosphate were widely used for sheep in Australia. Doses of 60 and 120 mg. fluorine had no effect on physical appearance of bone. Higher doses brought about replacement of normal colour by a white chalky appearance and the walls of the bone become thickened and in extreme cases exostoses appeared on the long bones. The mandible also showed exostoses and increase in diameter. Sheep receiving 60 mg. fluorine showed changes in the

teeth roughly proportional to the amount ingested. Fluorine intake caused incisors to erupt at an earlier age.

Peirce (1939) discussed the chronic fluorine intoxication in domestic animals. It is divided into observations of the disease as it occurs, (1) naturally e.g."gaddur" in Iceland and "darmous" in Morocco due to high fluorine content of the soil,herbage or water derived from natural sources, (2) due to the animals grazing in areas around the industrial concern and (3) due to experimental induced poisoning. Symptoms of the disease included dental changes (mottling of the teeth), emaciation, weakness, lamness and impaired locomotion associated with development of exostoses on the long bones and mandibles. Experimental observation had been reported in sheep. Young animals grow less rapidly and show general unthriftiness with marked chemical or pathological changes in the bones and teeth. The severity of the chronic intoxication depends upon the species and age of animals as well as upon the nature of the fluorine compound involved and duration and mode of administration.

Greenwood (1940) described the fluoride intoxication in animals. Fluorine was a general protoplasmic poison and could inhibit many enzymatic reactions. Acute poisoning was characterised clinically by salivation, nausea, vomiting, urination, muscular weakness, excitement, tremors, convulsions and lowering of blood

pressure. Death was due to inhibition of respiration. The acute toxic dosage was about 0.5 gm. per kg. body weight for mammals per os and 0.08 ± 0.15 gm. per kg. body weight on intravenous or subcutaneous administration. Mottling of the teeth was first detectable symptom of chronic fluorine intoxication following the consumption of water containing more than 1 p.p.m. fluorine, although such water were said to reduce the incidence of dental caries. Pathological changes in the kidney, thyroid and lymph-node had been reported.

Hotfield et al.(1942) described the effect of fluorine in rock phosphates in the nutrition of fattening lambs. The teeth of lambs ingesting highest amounts of fluorine showed discolouration but all the other lambs had excellent teeth. The fresh and dry weight of thyroid glands decreased but both the percentage and total content of iodine increased as the level of fluorine in the diet increased.

Spira (1942) described the effect of fluorosis on the parathyroid gland. He supported the hypothesis that the primary disturbance in chronic fluorosis is centred in the parathyroids and that mottled teeth, certain dermatosis and alpecia are secondary changes in tissues over which the parathyroids are said to exercise a regulatory function.

Shrewsbury et al.(1944) described the effect of fluorine in the nutrition of sheep. In the teeth of the sheep receiving 1.5 mg./kg. body weight daily, there was erosion of enamel.

Moule (1945) reported cases of fluorosis in sheep in Queensland. Water containing 2 parts per million of fluorine will produce symptoms of fluorosis in teeth of young animals.

According to Roholm (as quoted by Nicholson,1945) the lethal dose for sheep is 15 mg. per kg. of body weight.

Seddon (1945) described the cases of chronic endemic dental fluorosis occurring naturally in sheep in central Queensland. Water of artesian wells contained upto 19 parts per million fluorine. Mottling of the enamel, chalkiness and marked irregularity of wear were characteristic lesions in both incisor and molar teeth.

Boddie (1947) gave the details of the clinical signs observed in affected sheep. The dental changes involved mottling, hypoplasia and abnormal brittleness of the enamel of the permanent teeth of the animals grazing on contaminated pasture during the period of formation of permanent teeth.

Blakemore et al.(1948) reported the industrial fluorosis in farm animals in England, attributable to the manufacture of bricks, the calcining of iron stone and to enamelling process.

Delanoe (1948) correlated the experimental and natural fluorine poisoning. Fluorine is fatal in doses of over 68 mg. per kg. body weight experimentally but in districts where natural phosphates containing fluorine occur, animals and human beings developed resistance to the inhaled fluorine. Effects on temporary and permanent teeth were discussed.

Becmeur et al.(1951) reported the darmous and fluorine poisoning in Morocco. Calcium fluoride produced osteopetrosis, but the sodium fluoride caused osteoporosis.

Harvey (1953) reported fluorosis in Merino sheep. X-ray and chemical analysis indicated that effects of fluorosis on incisor teeth are permanent. Bone defects (rarefication and shortening of the horizontal ramus of the mandible) are not permanent.

Siddique (1955) reported cases of fluorosis of Nalgonda district in India. He described the clinical features of fluorosis in the inhabitants of 3 villages in Nalgonda district. Incidence of fluorosis has been reported from different places since 1933.

Herman (1956) discussed the fluorine poisoning in urinary tract calculi. Fluorine was found in high concentration in 8 out of 10 urinary tract calculi.

Liegeois et al.(1956) described the chronic fluorosis in sheep. The clinical symptoms and postmortem findings associated with chronic fluorine poisoning in 5 sheep were described. The main changes were in the bones and teeth. In the latter there was irregular wear on the molars giving the tables a wavy appearance. There was loss of enamel on the anterior surface of incisors which became dull and chalky. Significant changes were observed in the fluorine content of urine, bones and teeth.

Weatherell et al.(1959) described the skeletal changes of chronic experimental fluorosis. The gross and microscopic features of skeletal fluorosis in sheep to which sodium fluoride had been administered were described. Considerable haphazard exostosis developed upon mandibular and long bones.

Flatla (1962) reported the cases of industrial fluorosis in livestock in Norway. The total aluminium production in 1960 amounted to 1,89,700 tons about 5% of the world's production but as those are plans for stepping up production to about 800000 tons in the next 10 years, the magnitude of the problem of chronic fluorosis in livestock will steadily increase.

Faccini et al. (1965) attempted to see the effect of sodium fluoride on the ultrastructure of the parathyroid gland of sheep. Four pairs of twin lambs were used. One received

distilled water containing sodium fluoride, while its twin and the control received distilled water only. Slight pathological changes were observed after a month. Results suggested that the parathyroid glands were over active in severe skeletal fluorosis, but it was suggested that this could be due to early renal damage caused by fluoride.

Minciuna et al. (1966) reported the cases of industrial fluorosis of cattle and sheep in Roumania. This was the first report of disease in Roumania. It appeared in a herd of about 190 dairy cows and sheep grazing in the vicinity of a factory producing sulphuric and superphosphates. The clinical features included lameness, dental lesions and emaciations.

Tararov et al.(1968) reported the industrial fluorosis in cattle and sheep in Bulgaria. Fluorosis was established for the first time in Bulgaria in 1966. Fluorine was detected in herbage upto 5 kilometer from industrial works, its content varying from 18.4 to 391 parts per million dry matter. Cattle and sheep have severe dental lesions. The teeth of the sheep upto 2-3 years old were severely affected.

No published information is available on the haematological changes in sheep poisoned with sodium fluoride.

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MATERIALS AND METHODS

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EXPERIMENTAL SHEEP AND ITS MAINTENANCE:

The present research was conducted on 25 sheep of non-descript local breed. All the sheep were tagged with number and kept in same environment, management and food throughout the research. The standard ration comprises gram bhusi, salt, berseem grass and adlib supply of water. The sheep were kept in clean, dry and airy sheds which were cleaned daily and desinfected once in a week. Animals free from diseases and parasitic infestations were selected for fluorine poisoning.

BLOOD COLLECTION AND EXAMINATION:

About 5 c.c. of blood was collected aseptically with sterilised syringe from jugular vein of all the experimental sheep three days before the administration of the sodium fluoride to them. One drop of blood was dropped on clean glass slides and uniform smear was made for differential count of leucocytes and rest of the blood was transferred into a clean test tube containing 0.2 ml. of Wintrobe's isotonic anticoagulant for 5 ml. of blood. The Wintrobe's anticoagulant consisted of solution containing 2 % potassium oxalate and 3% ammonium

oxalate. It was evaporated to dryness in the tube on a water bath previously. The tubes were shaken for a few minutes to ensure a thorough mixing of blood and anticoagulant.

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

Total Erythrocyte count:-

Double Neubour's counting chamber was used for total erythrocyte and leucocyte count.

Hayem's solution was used as diluent for total erythrocyte count.

Total Leucocyte count.-

Double Neubour's counting chamber was used for total leucocyte count also.

Turk's fluid was used as diluent.

Preparation of staining of blood smears.-

Properly spread blood films made on polished and absolutely clear slides was used. Uniform smears thus made were stained by Leishman's staining technique. Two hundred leucocytes were counted in each slide following the "battlement" system

(1 m.m.down, 1 m.m. across and 1 m.m. above).

Haemoglobin determination.-

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

Erythrocyte sedimentation rate and packed cell volume.-

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

Clear Wintrobe's haematocrit tubes with a uniform of 3 m.m. bore and double 10 cm. scale caliberation with millimeter division were used. The scale on the left was read from top to bottom while the scale on the right was read in reverse. Tubes were filled with oxalated blood upto '0' mark on the left hand scale and allowed to stand vertically only. Only one reading was taken at the end of one hour. The Wintrobe's tubes were afterward centrifuged to determine the P.C.V. at speed of 3000 per minute for 1 hour and then for 30 minutes. The results were recorded as the number of ml. of cell per 100 ml. of blood.

Sodium fluoride administration.-

All the sheep were divided into three groups for producing poisoning.

1. First group.- This group was taken for producing acute fluorine poisoning. Five sheep were taken and poisoned with 15 mg./kg. body weight. Two sheep were kept as control animals in this group.

2. Second group.- This group is also taken for producing acute fluorine poisoning. Nine sheep were taken and poisoned with 0.5 gm./kg. body weight. Four sheep were kept as control animal in this group.

3. Third group.- This group was taken for producing chronic fluorine poisoning. Three sheep were taken and sodium fluoride was given in drinking water at the rate 20 parts per million. They were taking water adlib. Sodium fluoride was administered over a period of 5 months. But out of these two animals died after one month and two months respectively. There were two control animal in this group.

After administration of sodium fluoride these animals were kept in close observation. Blood from jugular vein with sterilized syringe were collected in test tubes for haematological tests half an hour before death and the analysis of blood was done as in normal cases as detailed above.

Postmortem examination.-

When the animals died due to poisoning,a postmortem

examination was adopted (Rubarth,1964). First the animals were examined externally. Colour of external mucous membrane of the body was noted. The carcases were opened and various parts of the body were examined systematically.

Histopathology.-

Pieces of organ which showed the macroscopic lesions were collected in 10% formaline for histopathological examination. After fixation for a few days, the small pieces of tissues were washed in running tap water for 20 hours, dehydrated in acetone, cleared in benzene and the blocks in paraffin were made. Sections were cut at 5 /u to 6 /u in thickness by hand driven microtome. These sections were stained by routine stain haematoxylin and eosin (Lillie,1954) and studied under microscope.

For decalcification of the bone formaline formic acid mixture was used (Culling,1957).

Statistical analysis.-

(1) The following formulae were used to obtain mean and standard error.

$$\text{Mean} = \frac{\sum_{i=1}^{n} xi}{n}$$ Where $\sum$ = Summation
n = Number of observation

$$S.E. = \sqrt{\frac{\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2 / n}{n(n-1)}}$$

Where S.E. = Standard error

$\sum$ = Summation

n = number of observation

(2) 't' test was done by conventional method as described by Panse and Sukhatme (1967).

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RESULTS

R E S U L T S

Sheep procured for experimental purpose were divided into three different small groups to facilitate easy execution of fluorine poisoning and recording of the haematological and tissue changes. Only those sheep which were free from diseases (parasitic infestation etc.) on three successive days were given sodium fluoride per os to produce intoxication.

Acute experimental poisoning (Group I).-

Sodium fluoride (15 mg/kg body weight) was given to produce poisoning in a group of 5 sheep against two controls. Sheep were destroyed after seven days of poisoning.

Symptoms.-

Sheep were dull and depressed. There was passage of thin watery nasal discharge in them. They did not take feeds for twenty hours after poisoning. They had starting rough coat and even avoided grazing and intake of water. They ran subnormal temperature ($98^{o}F$). After twenty hours, they took water and feeds. They even started grazing in the fields as usual. Later, they looked healthy and ran normal temperature. The animal of this

experimental group did not succumb to administration of sodium fluoride (15 mg.per kg.body weight) even within seven days. All of these were destroyed on seventh day and their organs were collected for histopathology.

Haematology.-

Different blood values (Red blood cell count, White blood cell count, Erythrocyte sedimentation rate, Haemoglobin percentage, Packed cell volume and Differential count) were estimated from second days onwards after poisoning (Table 2). There was significant increase in value of erythrocyte sedimentation rate on 3rd day after poisoning in the sheep (E.S.R. value - 35 mm. in sheep no.1). There was decrease in red blood cell count and haemoglobin percentage. There was slight increase in the percentage of neutrophils whereas no decrease was seen in the percentage of lymphocytes. There were no other significant changes in other blood values. All the sheep showed normal blood values on 7th day of poisoning.

Gross pathology.-

There were few subpleural reddened areas in the lungs. Several subcapsular greyish-white foci were present in the livers

of the affected sheep. The kidneys were slightly reddened and swollen. The intestinal mucosae were also slightly reddened and covered with blood tinged mucoid material. There was presence of clotted blood in the ventricles of heart. The meninges of the brain were moderately hyperaemic.

Histopathology.-

Lungs.- Blood vessels in peribronchial spaces were severely filled with blood. At places, the alveolar capillaries were slightly engorged with blood. The bronchi contained round cell and pink-stained material. There was increase in the size of lymphoid nodules in the peribronchial spaces. The arteries were thickened due to hyperplasia of muscle cells in media. The alveolar walls were slightly hyperaemic and pink-stained material was present in the alveoli. The interstitial tissue was more thickened and emphasized due to some round cell infiltration.

Liver.- Central veins of the livers contained excess of erythrocyte. The liver cells were swollen and more granular. They contained empty areas in their cytoplasm at places.

Kidneys.- The vessels in the interstitial tissue of the kidneys were engorged with blood. Interstitial tissue of the kidney was slightly thickened due to round cell infiltration.

Small intestine.- Many cells lining the crypts of Lieberkuhn were changed into goblet cells and were very much distended. There was also excessive number of goblet cells in the crypts. Epithelial cells lining the gland were desquamated to form clumps into the lumens. The lymphoid patches contained more dark pyknotic and almost hyalinised areas (Fig.1).

Heart.- Cardiac musculature has lost the striation. There was fragmentation of cardiac muscle fibres at places. They contained granular material.

Brain.- There were several dark stained microglia around the neurons. Nucleus had got eccentric position in the neurons.

Autopsy of the control animals did not reveal any changes in them.

Acute experimental poisoning (Group II).-

Sheep in the second experimental group were administered sodium fluoride (0.5 gm. per kg. body weight) per os. Some of these sheep died even after an hour of poisoning whereas others died at variable periods (Table 3). One of these sheep survived for more than 5 days after poisoning and it gradually reverted to normal. Its different blood values were normal. It

was killed on seventh day of poisoning.

Symptoms.-

Affected animals became dull and depressed. They showed frequent nervous excitement and frequently stretched their heads. They were restless and lied on the ground and stood on their legs at times. There was profuse salivation and watery lachrimation. There was passage of blood tinged discharges from the nares and temperature was subnormal in them (Table 3). They were excreting loose blood tinged faeces at frequent intervals. There was scanty urination in them. Hurried difficult breathing was seen in the affected sheep. While lying on the ground, they pressed their heads on the sides of their bodies.

Haematology.-

The data on total Red blood cell count, total White blood cell count, Haemoglobin percentage, Erythrocyte sedimentation rate, Packed cell volume and Differential count of the leucocytes were subjected to 't' test to see significant difference between the experimental and control group. The 't' test revealed that there was highly significant difference in total Red blood cell count, Haemoglobin percentage, Erythrocyte sedimentation rate,

Packed cell volume, Eosinophil percentage and there was no significant difference in total White blood cell count, Monocyte, Lymphocyte and Neutrophil percentage (Table 3).

Gross pathology.-

There were a few subpleural depressed areas below the level of adjacent tissue into the lungs. There were also raised greyish white subpleural areas (Figs.2 & 3). There was presence of regurgitated ingesta in the trachea and bronchi. Blood clots were present into the trachea. Livers of the affected sheep were severely swollen and reddened. Blood was flowing from the cut surfaces of the liver. The gall bladder was distended with bile. The edges of the livers were round. Kidneys were swollen and pale. Urinary bladder was empty. Abomasal mucosa was reddened and hyperaemic and contained blood stained mucoid material in two cases(Fig.4). The ruminal papillae were severely reddened in patches. Reddened patches (about 6" in diameter) were frequently seen close to esophageal groove in the rumen. These lesions were seen in all sheep except two. These two animals died after 72 hours of poisoning. Reddening of the mucosa of small intestine was seen in seven cases. Mesentric veins were engorged with blood. Spleen was slightly swollen. The blood tinged material was present

on its cut surfaces. Trabeculae and Malpighian corpuscles were not visible. The right ventricle was severely dilated and a groove was present between right and left ventricles. The musculature of the right ventricle was soft and pliable. There was presence of unclotted blood in the ventricles of heart. Meninges of the brain were slightly hyperaemic. Adrenal glands were moderately swollen and slightly reddened. Thyroid glands were slightly swollen.

Histopathology.-

Lungs.- There were areas of atelectasis and emphysema. There were small focal areas where alveolar walls were very much approximated to each other to leave behind narrow slits. Many alveoli were very much distended and even broken alveolar walls communicated to each other (Fig.5). The vessels in the peribronchial spaces were highly hyperaemic.The bronchi contained eosinophilic material with nuclear debris. The vessels in the alveolar walls were hyperaemic. The bronchi were very much distended. The bronchioles contained exudate consisting of neutrophils and lymphocytes etc. The alveoli were filled with pink stained eosinophilic material in the sheep dying on the 4th day after poisoning (Fig.6). The capillaries in the alveolar

walls were highly engorged with blood. The bronchi and bronchioles contained pink stained material along with few epithelial cells. There was presence of hyperaemia and oedema.

Liver.- The vessels in the portal tract were hyperaemic. Epthelial cells were highly hyperchromatic throughout the lobule. Some of them contained vacuoles. The cell boundries were indistinct. The liver cells were swollen and showed vacuolic degeneration. Sinusoids contained excess of erythrocytes (Fig.7). The central veins were engorged with blood. The cells in the hepatic cords were highly eosinophilic throughout the lobule and contained pyknotic nuclei at places. Epithelial cells had lost their nuclei at places. There was pink stained material in the interlobular spaces.

Kidney.- Many of the tubules contained an almost homogeneous material along with few nuclei. The tubules were lined with highly eosinophilic cells which were showing degenerative changes. Many of them were vacuolated, enlarged and contained pyknotic nuclei. Bowman's capsule was slightly widened and contained pink staining material. The tubules contained pinkish material with a few nuclei. Epithelial cells lining the tubules had lost their boundries and contained almost homogeneous granular material (Fig.8). The blood vessels in the

interstitial tissues were engorged with blood. The vessels in interstitial tissue contained eosinophilic matterial (oedema). The epithelial cells lining the tubules were desquamated to form eosinophilic granular material in the lumens of tubules. Epithelial cells lining the tubules were very much swollen and had almost obliterated lumens of the tubules.

Abomasum.- The epithelial cells lining the glands were desquamated to form clumps into the lumens. Lumens of the glands of stomach had contained dark nuclei at places. Degenerative changes were present. The epithelial cells lining certain glands in the lamina propria were separated from each other by empty spaces. The cells of the gland had got pyknotic nuclei and contained eosinophilic material in the lumens. The cells of the lamina epithelialis of the abomasum were highly eosinophilic with unusually round and hyperchromatic nuclei.

Rumen.- The cells of the epithelial layer had got hyperchromatic and pyknosed nuclei. The subepithelial capillaries and blood vessels in the lamina propria were severely engorged with blood (Figs.9 & 10).

Small intestine.- Brunner"s gland had got widened lumens and contained many mononuclear cells and lymphocytes. The crypts cell had got hyperchromatic nuclei. The lumen was filled

with eosinophilic material. The Brunner's gland contained eosinophilic material with a few nuclei. The lining epithelial cells of the crypts of Lieberkuhn were swollen and desquamated with pyknotic nuclei. The vessels in the lamina propria were excessively filled with erythrocytes (Fig.11). There were areas of focal haemorrhages in the lamina propria. There was pink stained eosinophilic material into the submucosa. The epithelial cells lining the crypts of Lieberkuhn were highly eosinophilic and desquamated to form clumps into the lumen. The cells in the lamina propria were hyperchromatic and pyknotic. The lamina epithelialis was almost lost. The cells in the lymphoid follicles were very much pyknotic. The cells in the gland of lamina propria had been desquamated to form clumps in the lumens of the gland. The vessels in the lamina propria were hyperaemic. There was pink stained material in the submucosa. The epithelial cells of the villi were more granular and swollen and had vacuolated appearance.

Spleen.- There was excess of erythrocyte in the red pulp (hyperaemia). Malpighian corpuscles were numerous and large in size. Lymphoid cells at the centre of the follicle were more pale stained than those at the periphery of the follicle and there were also such numerous corpuscles.

Heart.- There was loss of striation in muscle fibres. The fibres contained eosinophilic granular material. The blood vessels in the heart were hyperaemic. There were areas of focal haemorrhages. The nuclei are hyperchromatic and fibres are broken at places (Fig.12). Dark stained pyknotic nuclei were present.

Brain.- Blood vessels were hyperaemic. The cells of neurons were located peripherally. Perineural and perivascular spaces were widened (Fig.13). There were several empty spaces of different shapes and sizes. These empty spaces contained homogeneous material at places. Glia cells were present at the margin of these empty spaces. Several glia cells are found around the degenerative neurons and have even entered into the cytoplasm of the neurons. Neuronophagia and satellitosis were present in these cases.

Adrenal glands.- The cells in the cell cords of zona fasciculata were swollen. They had got granular cytoplasm with more vacuolation. There were irregular spaces within the gland cells. There was an excess of erythrocyte in sinusoids of medulla (Fig.14). There was presence of pink staining material in between the cell cords (oedema). Such pink stained material was also present in between the columns of cells of

zonafasciculata of cortex (oedema). Cells in cords were very much disorganised at places. The vessels in the cortex of adrenal were hyperaemic. The epithelial cells of zonafasciculata were swollen, more granular and vacuolated. There were irregular spaces in the cells of zonafasciculata. There were areas of haemorrhage into the cortex. The cells of zonafasciculata had got irregular empty spaces in the cytoplasm. The cells of the medulla and cortex were swollen. They had got granular cytoplasm.

Thyroid glands.- The vessels in the interstitial tissue were filled with blood. Most of the follicles were filled with eosinophilic homogeneous colloid whereas in other, there were several very conspicuous homogeneous acidophilic spherules (Fig.15).

Autopsy of the control animal did not reveal any changes.

Chronic experimental poisoning (Group III).-

Three sheep were given 20 parts per million fluoride against two control sheep.

Symptoms.-

Of these three animals, two died after one and two months respectively. There was respiratory distress in them and

they were under poor nutritional condition. The conjunctiva of the eyes were pale. The last animal of this group was killed after five months. There was no any abnormal symptoms noticed in this sheep. It was having fair nutritional condition.

Haematology.-

Changes were noticed only in differential count of the affected sheep. There was increase in the percentage of lymphocytes and decrease in that of neutrophils (Table 4). Erythrocyte sedimentation rate was also slightly increased.

Gross pathology.-

Lungs of the first two animals were slightly reddened and blood was flowing on the cut surfaces. There were several hydatid cysts. There was also areas of consolidation. Kidneys were swollen and pale. Small intestine was slightly hyperaemic. Gall bladder was severely enlarged and elongated. Spleen and thyroid glands were moderately enlarged.

Histopathology.-

Kidneys.- Epithelial cells lining the tubules were slightly swollen and the interstitial tissue was slightly emphasised due to dirty pink stained material (Interstitial oedema).

Small intestine.- Epithelial cells lining the crypts of Lieberkuhn were more swollen and almost filling the lumens. There were lymphocytic infiltration in the lamina propria.

Spleen.- Lymphoid corpuscles were present more than usual. Lymphoid cells were deeply stained and had very little cytoplasm.

Thyroid glands.- The blood vessels of the thyroid glands were hyperaemic. Thyroid glands contained pink stained material.

Autopsy of the controls in this group did not reveal any changes.

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Table I

Blood values of normal sheep before administration of sodium fluoride.

No. of animal		Temp. °F	R.B.C. x 10^6	W.B.C. x 10^3	Hb. gm. %	E.S.R. mm./hr	P.C.V. c.mm./100cc.	Differential count				
								Mono-cyte %	Lympho-cyte %	Neutro-phil %	Baso-phil %	Eosino-phil %
	Maximum	103.0	11.00	11.80	11.5	1	33	6	68	50	2	10
	Minimum	102.0	7.06	6.89	8.4	0	24	2	40	22	0	3
25	Mean	102.63	8.76	9.03	10.23	0.12	27.32	3.60	58	38.8	0.12	6.76
	Standard error	±0.02	±1.55	±0.52	±0.53	—	±0.49	±0.12	± 2.23	± 1.86	—	± 0.33

Table 2

Blood values of poisoned sheep (Group I) after administration of sodium fluoride

No. of animal		Temp. °F	R.B.C. x10^6	W.B.C. x 10^3	Hb. gm. %	E.S.R. mm./hr.	P.C.V. c.mm./100cc.	Differential count				
								Mono-cyte %	Lympho-cyte %	Neutro-phil %	Baso-phil %	Eosino-phil %
5 (poisoned)	Maximum	101.00	7.20	10.70	9.40	35.00	26.20	5	53	54	1	9
	Minimum	99.50	6.60	8.20	8.50	2.50	24.00	3	35	34	0	6
	Mean	100.30	6.83	8.38	9.20	13.50	25.40	4.2	46.8	41.6	—	7.1
	S.E.	± 0.25	±0.13	±0.26	±0.14	±5.03	± 0.93	± 0.14	± 3.89	±3.91	—	± 0.8
2 (controlled)	Maximum	102.60	9.42	8.95	10.60	0	28.00	4	56	37	0	6
	Minimum	102.50	8.85	7.00	10.20	0	27.00	4	53	35	0	5
	Mean	102.55	9.13	7.57	10.40	—	27.50	4	54.5	36	—	5.5
	S.E.	± 0.14	± 0.38	± 0.81	±0.28	—	± 0.71	—	± 1.5	± 1	—	± 0.5

S.E. = Standard error.

Table 3

Blood values of poisoned sheep (Group II) after administration of sodium fluoride

No. of animal		Temp. °F	R.B.C. x 10^6	W.B.C. x10^3	Hb. gm. %	E.S.R. mm./hr.	P.C.V. c.mm./100cc.	Differential count				
								Mono-cyte %	Lympho-cyte %	Neutro-phil %	Baso-phil %	Eosino-phil %
9 (Poisoned)	Maximum	100.00	8.28	11.00	10.00	5.00	28.00	6	63	45	0	8
	Minimum	96.50	4.78	6.74	7.00	1.00	23.00	3	46	26	0	2
	Mean	97.50	7.46	9.11	8.27	2.10	26.61	3.55	55.11	36.66	—	4.55
	S.E.	± 0.36	± 0.40	± 0.55	±0.36	± 0.44	± 0.52	± 0.24	± 3.43	± 2.46	—	± 0.44
4 (Controlled)	Maximum	103.00	10.98	9.82	11.40	0	34.00	4	65	33	0	9
	Minimum	102.50	9.50	6.90	10.50	0	30.00	3	60	26	0	5
	Mean	102.77	11.18	8.26	11.02	0	31.25	3.25	64.42	28.50	—	7.50
	S.E.	± 0.11	± 0.43	± 0.59	±0.77	0	± 0.88	± 0.25	± 0.98	± 1.65	—	± 0.33
Mean difference		5.27	2.72	0.85	2.75	2.1	4.64	0.30	9.31	8.16	—	2.95
Calculated 't' value		-	3.737^{**}	0.929^{NS}	3.773^{**}	3.134^{**}	4.768^{**}	0.75^{NS}	1.776^{NS}	2.071^{NS}	—	4.172^{**}

S.E.= Standard error; * = significant($P < .05$); ** = Highly significant ($P < .01$);

NS = Not significant.

Table 4

Blood values of poisoned sheep (Group III) after administration of sodium fluoride

No. of animal		Temp. °F	R.B.C. x10^6	W.B.C. x10^3	Hb. gm. %	E.S.R. mm./hr.	P.C.V. c.mm/100cc.	Differential count				
								Mono-cyte %	Lympho-cyte %	Neutro-phil %	Baso-phil %	Eosino-phil %
3 (Poisoned)	Maximum	102.00	9.00	8.99	10.80	2.5	28.00	4	62	30	0	9
	Minimum	99.50	7.25	7.20	9.00	0.5	26.00	3	59	25	0	6
	Mean	100.50	8.18	7.97	9.60	1.5	27.33	3.66	60.33	28.33	—	7.33
	S.E.	± 0.76	± 0.53	± 0.79	± 0.96	± 0.5	± 0.25	± 0.33	± 0.54	± 1.64	—	± 2.23
2 (Controlled)	Maximum	102.80	8.89	7.85	10.50	0	28.00	4	68	31	0	8
	Minimum	102.50	8.18	7.30	10.50	0	28.00	4	58	28	0	7
	Mean	102.65	8.80	7.77	10.50	—	28.00	4	65	29.5	—	7.5
	S.E.	± 0.72	±0.26	± 0.15	—	—	—	—	± 2.5	± 1.5	-	± 0.5

S.E.= Standard error.

D I S C U S S I O N

D I S C U S S I O N

Perusal of the pertinant literature went to show that there are little informations available on the pathologic changes of acute fluorine poisoning in sheep. However,certain informations could be found on the chronic fluorine poisoning (Fluorosis) in sheep. From results of the present studies, certain facts appeared which might be valuable in elucidation of the pathogenesis of fluorine poisoning.

At first acute fluorine poisoning was produced in a group of 5 sheep by administering sodium fluoride 15 mg./kg. body weight per os. Results obtained after poisoning were compared with those of controls and as well as the values of the same sheep estimated before the administration of poison. The sheep in the first group showed signs of improvement from second day of poisoning and become almost normal after 5th day. The different blood values taken in them on 7th day were in normal range. On the third day there was well marked increase in erythrocyte sedimentation rate (Table 2) and fall in red blood cell count in all the sheep of this group. According to Roholm (1937), acute toxic dose of sodium fluoride is 15 mg./kg. body

weight in sheep. But the results obtained in the present studies are at variance with his findings on this subject.

The toxic dose of 0.5 gm. per kg. body weight was adopted for producing acute poisoning in the sheep of the 2nd group and the results obtained were more or less in accordance with studies of Greenwood (1940). The main pathologic lesions in the sodium fluoride poisoning in sheep were those of gastro-enteritis, hepatitis, and nephritis. Several hyperaemic patches in the ruminal wall were the most conspicuous lesions in the affected sheep (about 78%). There were no significant pathologic lesions in the organs of one sheep killed on 7th day. Only one sheep in this group did not die before 6th day after poisoning.

The symptoms observed in the second group of sheep given 0.5 gm./kg. body weight were frothy salivation, profuse watery lachrimation and nervous excitement. They passed very little urine. There was blood tinged discharge from the nares. Temperature was subnormal. Greenwood (loc.cit.) described salivation, nausia, vomiting, excitement, convulsion and lowering of blood pressure in the animals. Inhibition of respiration was incriminated to cause death in animals. Areas of atelectasis, emphysema and dilatation of right ventricle were important lesions in the sheep dying of acute fluorine poisoning. In the

present studies, deaths of sheep appear to arise from acute dilatation of heart and collapse in lungs. There were areas of degenerative changes such as oedema, hyperaemia,atelectasis and neuronophagia in the brain of sheep. Nervous disturbances would have been caused in sheep suffering from acute fluorine poisoning due to degenerative changes in the brain.

Hyperaemic patches were noticed in the rumens in most of the cases (7 out of 9 sheep). Papillae were deep red and had red valvate like appearance. The author is not aware of any description of such lesions in literature. Smith and Jones(1966) have described lesions of acute gastroenteritis in fluorine poisoning in animals. The affected animals showed increase in erythrocyte sedimentation rate. It seems that there has been no work on this aspect of haematological change in fluorine poisoning in ovines. There was conspicuous fall in the red blood cell count and haemoglobin percentage in sheep of both acute and chronic group.

The sheep of third group were given 20 parts per million sodium fluoride over months. The conspicuous changes were noticed in the spleen and gall bladder. Splenic corpuscles were very much enlarged and prominent. Spleen was very much swollen and enlarged with round edges. The gall bladder was

severely elongated and highly distended with bile. Red blood cell had decreased values whereas erythrocyte sedimentation rate was slightly increased. Pandey and Lall (1946) carried out haematological studies in cattle and their main haematological findings were decrease in the number of erythrocytes, haemoglobin content and predominance of lymphocyte over neutrophils. In the present studies, similar haematological findings were also noticed. There was decrease in the number of erythrocytes, haemoglobin content and predominance of lymphocytes over neutrophils. At this stage it is also pertinant to mention that lymphocytes showed increased values in chronic cases whereas neutrophils predominated over lymphocytes in acute cases of the first experimental group.Thus haematological picture in acute and chronic cases differs from each other in some respects. Hyperchromic anaemia was produced in all these three groups of experimental sheep given fluorine intoxication.

No gross and microscopic lesions were noticed in the sheep even after daily intake of 20 parts per million for 5 months. Attrition and mottling of the teeth were not seen.

Survey of incidence of fluorine poisoning was conducted in the villages on foot hills of Rajgir. Gross examination of one thousand sheep for attrition and mottling of teeth and also

presence of exostosis was conducted. No sheep showed lesions of fluorosis. According to pioneer work on fluorosis in India by Shortt and his collaborators (1937), Majumdar, Ray and Sen(1943, 46), Pandey and Lall (1944,46), Mariakulandai and Ramiah(1941,45) fluorosis in man and animals is prevalent in certain areas of India. Fluorosis zones are situated at an attitude of between 600-1200 ft.of sea level mostly in the vicinity of mountain ranges. Sahai (1937-38) reported probably cases of fluorosis in Bihar. It is apparent from the results obtained in Rajgir area that there is still the need of detailed and thorough investigation for finding out the extent of fluorosis problem and confirmation of the clinical cases of fluorosis in animals in Bihar.

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S U M M A R Y

SUMMARY

In the present studies fluorine poisoning was produced in three groups of sheep. Administration of sodium fluoride 15 mg./kg. body weight per os to the sheep in the first group failed to produce deaths in any sheep of this group. However, there was increase in erythrocyte sedimentation rate and percentage of polymorphonuclear leucocytes in the sheep of this group. Red blood cell count and haemoglobin percentage was less than normal in the affected sheep. These findings do not support the work of Roholm (1937) about the toxicity of sodium fluoride at the dose of 15 mg. per kg. body weight. Gross and microscopic changes have been described in animal of this group.

The animals in second group were given 0.5 gm.sodium fluoride per kg. body weight. There was conspicuous fall in red blood cell count and haemoglobin percentage in the affected animals. Large reddened patches were very remarkable gross lesions in the rumen of the sheep in this group of acute fluorine group. Rumen papillae were severely swollen and reddened. There appears to be no description of such lesions in literature in sheep in fluorine poisoning. Pathologic changes in the different organs of the dead or killed sheep have been described.

The animals in the chronic group were given 20 parts per million sodium fluoride. None of the sheep developed lesions in teeth and bones of their bodies.

Some survey of the incidence of fluorine poisoning was carried out in one thousand sheep maintained by shepherds on the foot hills of Rajgir (Bihar). No sheep with gross lesions of fluorosis in the teeth and bones could be detected.

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PLATE - I

Fig.1. Section of small intestine of a sheep, showing necrotic changes in the lymphoid follicle.
H & E stain x 100. Sheep no.1

Fig.2. Emphysematous patches in the lungs of a sheep affected with fluorine poisoning.

Sheep no.8.

PLATE - II

Fig.3. Atelectatic areas in the lungs of a sheep affected with fluorine poisoning.

Sheep no.11.

Fig.4. Marked hyperaemic changes in the abomasal mucosa of a sheep affected with fluorine poisoning.

Sheep no.10.

PLATE - III

Fig.5. Section of a lung of a sheep showing abnormally distended and intercommunicating alveoli.

H & E stain x 100. Sheep no.16.

Fig.6. Section of a lung of a sheep showing hyperaemic and oedematous changes.

Note.- Oedema fluid with a very few cells in the alveolar spaces.

H & E stain x 100. Sheep no.14.

PLATE - IV

Fig.7. Section of a liver of a sheep showing distended sinusoids containing excess of erythrocyte and somewhat disorganised epithelial cells in cords.

H & E stain x 400. Sheep no.16.

Fig.8. Section of a kidney of a sheep showing almost homogeneous gramular material in the tubular lumen and somewhat slightly pink stained material in the bowmen's capsule.

H & E stain x 400. Sheep no.15.

PLATE - V

Fig.9. Section of a rumen showing hyperaemic changes in the subepithelial vessels in the lamina propria.

H & E stain x 400. Sheep no.14.

Fig.10. Hyperaemia in the subepithelial vessels of the lamina propria of a rumen of the sheep.

H & E stain x 400. Sheep no.13.

PLATE - VI

Fig.11. Section of intestine of the sheep showing focal haemorrhages in the lamina propria and degenerating changes in the crypts.

H & E stain x 400. Sheep no.14.

Fig.12. Section of a heart showing fragmentation of muscle fibres and disappearance of cross striation in a sheep.

H & E stain x 400. Sheep no.9.

PLATE - VII

Fig.13. Section of cerebrum of a sheep showing hyperaemia and perivascular oedema.

H & E stain x 400. Sheep no.16.

Fig.14. Section of adrenal gland of a sheep showing hyperaemia in the medullary portion.

H & E stain x 400. Sheep no.17.

PLATE - VIII

Fig.15. Section of thyroid gland showing several homogeneous spherules in the follicles.

H & E stain x 400. Sheep no.15.

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