Vet.Med.J.,Giza. Vol.42,No.1.(1994):295-304

PICTURE OF ENDEMIC FLUROSIS IN HENS

BY

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Received :

SUMMARY

The present study was carried out to investigate the picture of exposure to fluoride in hens nearby superphosphate fertilizer producing factory at Manquabad, Assiut governorate, Egypt.

Twenty hens selected from farmer's houses showing some skeletal deformity were investigated in this study.

The gross appearance of bone revealed hyperossification and deformity. Bone analysis showed an increase in dry matter %, Ash % and Ca content.

Fluorine, thyroxine, Triodothyronin and iodine levels showed an apparent increase in serum. Red blood cells, packed cell volume and haemoglobin content showed also an elevation than control hens. Total leucocytic count was decreased in investigated hens. Albumin/globulin ratio showed a decreased values as well as total proteins and calcium in serum.

Tissue samples of liver, Kidney, lung, heart and thymus were showing a necrobiotic and degenerative changes.

INTRODUCTION

Poultry considered one of the main source of income and production of animal proteins in Egyptian villages. The recent increase in Litrature on the use of fluorides in the treatment of certain poultry and human metabolic bone diseases, particularly osteoporosis, has prompted the present study to determine the incidence of bone diseases particularly skeletal fluorosis and clarify the picture of fluorosis in random samples of incivioual hens rearing an endemic polluted area at Manquabda village. Approximately 99% of the fluorine (F) retained in the normal body is stored in the bone resulting in bony changes Ramberg and Ulson, (1970). The use of Rock pnospnate as calcium and phosohorus supplementation inocuced dietitic problems through fluoride exposure. The great variety of skeletal deformity occuring from many other causes press this work to identify and exclain the picture of endemic fluorosis in hens. This study was conducted to threw some light on the biological effect of fluorides in system of hens.

MATERIALS AND METHODS

Twenty balady hens, each of them weighting 900-1100 gm. acing from 10-13 months were selected from El-Tawabia Village which lies nearby Manguaad superphosphate producing plant. Hens showed some skeletal defects they were subjected to our examination control hens were chosen from an area faraway from any source of pollution (Manfalout, 18 Km southern of the factory)

Blood samples were obtained from wing veins to conduct the haematological picture, serum was obtained for estimation of fluorine, calcium, phosphorus. iodine, triiodothyronin, thyroxine, gamma glutamyl transaminase, total proteins and albumine/globulin ratio. Hens were slaughtered and tissue samples for analytical and histopathological survey were taken. Bone was inspected for any abnormality.

Red blood cells (RBCs), white blood cells (WBC), Haemoglobin (HB), packed cell voume (PCV). Mean corpuscular voume (MCV), Meancorpuscular haemoglobin concentration (MCHC) were detected according to methods included in Coles (1986).

Total protein and albumin were determined in serum acording to Weichselbaum (1946) and Drupt (1974) respectively. Fluorine, calcium, phosphorus, and lodine were determined after Fry nad Taves (1970), Bett and Fraser (1959), Mornil and Prox (1973) and Morin et al. (1975) respectively. Gama G. T. activity was determined acording to the method of Szasz (1969). Quantitative determination of T3 and T4 in serum were obtained after Zoasoo (1975), Statistical analysis of data was performed after Kalton (1967).

RESULTS

The results obtained for fluorine, calcium, phosphorous, Gama Glutamic Transaminas (F, Ca, Ph, & G.T) haematological picture; and differential leucocytic count. Total proteins, ilustrated in tables 1, 2 and 3, Albumine, globulin nad albumin/globulin ratio are shown in table (4) electrophorotic pattern; T3, T4 and iodine (1) in serum are present in (tables 5 and 6). Bone analysis for fluorine, dry matter% Ash% calcium and phosphorus are present in table 7.

The investigted chosen hens were appare healthy except some deformities in hen's l Postmortem examination revealed congestio some areas of lung tissue adjacent to scatte emphysematous areas in most cases. The li appeared pale and yellowish in all cases w kidneys appeared swollen (bright cloour) an darker in colour. The heart in all cases appeare normal/size while cardiac muscles show multible areas of paleness in most cases. Thy appeared smaller in size in most cases but in so ones enlarged lobules were detected. Bo wxamination after cleaning showed roughnes the external surface and sclerotic changes wh appeared in the scierosis of end attachments tendons and musculature, increased growth tubercles and eminences of bone (Fig., 2) as i ca of ribs and ilioischial junctions.

Bone fractures had been recorded in clavic where callus formation indicates this previo fractures (Fig. 1). The over growth of bones a the changs of cartilagenous areas to an irregul and uneven uncontrolled growthes acarent appeared in sternum (Fig. 1).

Histopathological changes appeared to be parenchamatous type in addition to the dire effect on bones, liver showed mild to modera

Table (1): Fluorine, Ca. P and GGT. levels in serum of investigated hens

Hens		Fluorine (PPM)	Calcium mg%	Phosphorus mg%	GGT. (I.U.)
Investigated	Meam ± S.E.	0.788 ± 0.059**	8.577 ± 0.237**	10.228 ± 0.44***	20.696 ± 0.537**
	Average	0.33 - 1.70	5.172 - 10.656	6.55 - 14.05	12.05 - 26.96
Control	Mean ± S.E.	0.028 ± 0.002	10.653 ± 0.170	8.35 ± 0.424	30.896 ± 1.803
	Average	0.008 ± 0.050	9.030 ± 11.494	8.55 - 12.35	13.45 - 54.05

S.E. = Standard error
* = Significant at P < 0.05
** = Significant at P < 0.01

Table (2): Haematological picture in hens at endemic area of fluorosis

Hens		RBCs X106/UL	PCV %	Hb g/dL	MCV Cu/u	MCH U/ug	MCHC %	WBCs (10)3
Investigated	Mean ± S.E.	3.89 ± 0.16**	34.10 ± 0.43**	12.57 ± 0.24**	78.12 ± 2.33**	31.29 ± 1.11**	26.72 ± 0.66**	12.18 ± 0.31
	Average	2.48 - 6.10	28.00 - 38.00	10.5 - 16.0	62.29 - 111.94	18.72 - 34.75	20.89 - 33.33	8.00 - 18.00
Control	Mean ± S.E.	2.93 ± 0.07	31.14 ± 0.41	11.7 ± 0.15	118.32 ± 3.16	41.01 ± 1.17	30.27 ± 0.55	13.83 ± 0.45
	Average	2.47 - 4.14	28.00 - 35.00	9.00 - 13.00	83.33 - 153.84	32.73 - 52.63	25.45 - 35.22	10.00 - 18.00

S.E. = Standard error
** = Significant at P < 0.01

Table (3): Differential leucocytic count in hens at endemic fluoresed area

	Monocyte	Basophil	Eosinophil	Hetrophil	Large lymphoc	Small L. cyte
Investigated	0.983 ± 0.082*	2.909 ± 0.115**	1.086 ± 0.076**	8.043 ± 0.142**	1.647 ± 0.123**	84.272 ± 541**
Control	1.268 ± 0.061	5.134 ± 0.179	5.134 ± 0.179	4.966 ± 0.246	8.166 ± 0.540	80.33 ± 0.90

S.E. = Standard error
** = Significant at P < 0.01

Table (4): Total protein, albumin and globulin levels in investigated hens

Hens		Total protein g/L	Albumin g/L	Globulin g/L	Alb.Glob. ratio
Investigated	Meam ± S.E.	5.002 ± 0.68**	1.29 ± 0.052**	3.914 ± 0.063	0.33 ± 0.027**
	Average	4.770 - 6.752	1.95 - 2.40	2.431 - 4.25	0.22 - 0.68
Control	Mean ± S.E.	6.869 ± 0.108	2.422 ± 0.098	3.876 ± 0.065	0.62 ± 0.02
	Average	4.770 - 7.232	1.510 - 3.515	2.350 - 4.350	0.34 - 0.62

S.E. = Standard error
* = Significant at P < 0.05
** = Significant at P < 0.01

Table (5) Electrophoretic pattern of investigated hen's serum (mean ± S.E.)

Hens		Albumin %	Globuline %		
			Alpha	Beta	Gamma
Investigated	Meam ± S.E.	25.86 ± 2.01**	13.02 ± 0.95**	9.02 ± 0.63**	52.16 ± 3.25**
Control	Mean ± S.E	35.25 ± 2.25	22.11 ± 2.03	16.17 ± 1.02	26.49 ± 1.86

S.E. = Standard error
* = Significant at P < 0.05
** = Significant at P < 0.01

Table (6) :T3, T4 and iodine leveles investigated hens

Hens		Alpha	Beta	Gamma
Investigated	Meam ± S.E.	1.675 ± 0.128**	10.25 ± 0.096**	0.176 ± 0.006**
	Average	1.30 - 2.10	10.00 - 10.50	0.098 - 0.280
Control	Mean ± S.E.	0.850 ± 0.096	4.333 ± 0.437	0.091 ± 0.004
	Average	0.60 - 1.10	2.20 0 7.00	0.023 - 0.140

S.E. = Standard error
* = Significant at P < 0.05
** = Significant at P < 0.01

Table (7): Dry matter, Ash% Fluorine, Calcium and phosphorus percentage in investigated hens

Hens		Drymatter %	Ash %		F ppm	Ca %	P %
			(Wet base)	(Ury base)			
Investigated	Meam ± S.E.	0.799 ± 0.004***	0.403 ± 004**	0.490 ± 0.005**	5168 ± 260.51	44.382 ± 0.313**	27.90 ± 0.499
	Average	0.780 - 0.810	0.390 - 0.410	0.48 - 0.51	3486 - 7362	41.419 - 48.858	22.70 - 32.80
Control	Mean ± S.E.	0.725 ± 0.011	0.352 ± 0.006	0.448 ± 0.009	781.98 ± 25.88	35.288 ± 0.40	27.866 ± 0.123
	Average	0.70 - 0.76	0.340 - 0.37	0.422 - 0.465	587.60 - 949.20	32.97 - 36.57	27.30 - 28.50

S.E. = Standard error
* = Significant at P < 0.05
** = Significant at P < 0.01

Showed
A: progressive nswely bone formation in sterun (uncontrolled growth).
B. Fractured ribs with callus formation.
C. Fractured clavicle with callus form.

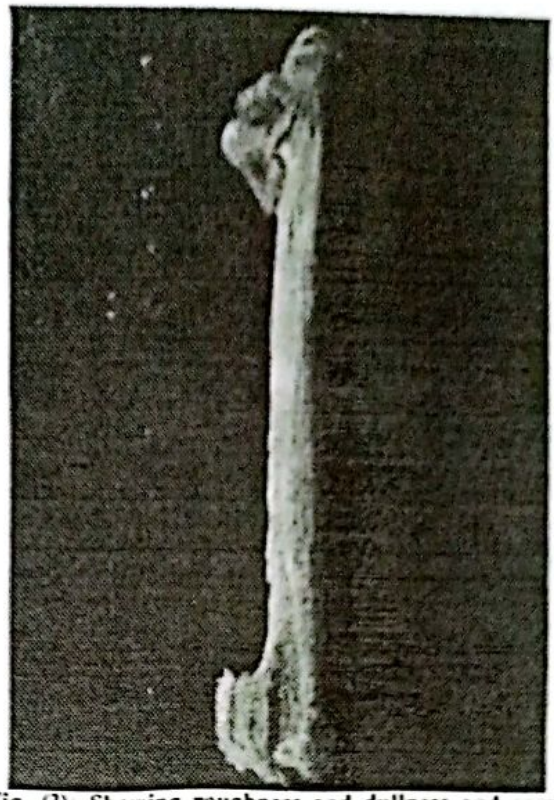
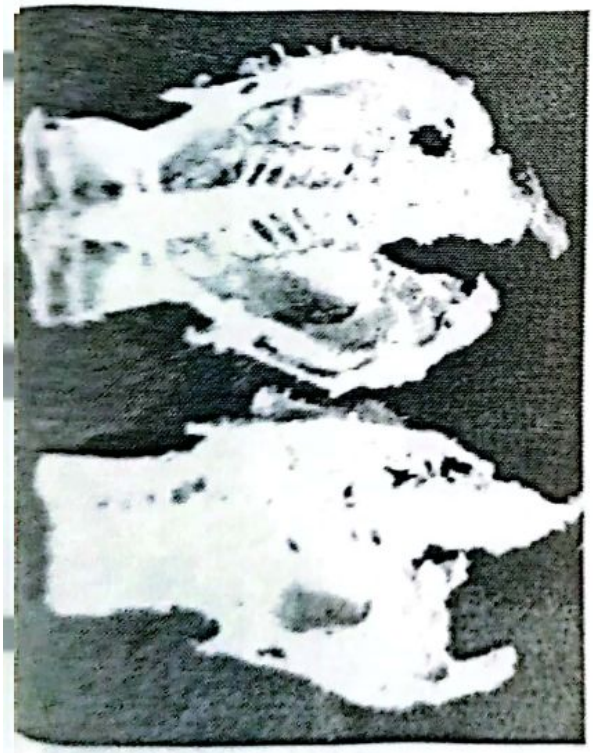


Fig. (3): Showing roughness and dullness and over grothes at the tendenus junctions of tarsometa tarsa bone.



Over groth and thickening of the ilioischial junction.

Fig. (4): Hepatocyte showed degeneration nd necrobiotic changes. congestion of central veins and mononuclear cell infiltration.

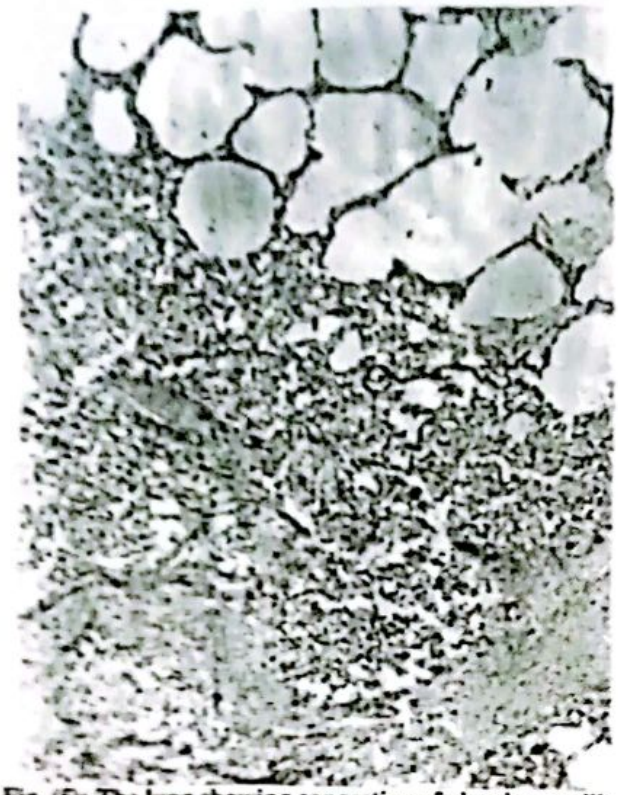


Fig. (5): The lung showing congestion of alveolan capilla ries, emphesema nad mono nuclear cell infiltration.

Fig. (7): Moycardium showed focal degeneration and lative necrosis and focal area of inflammat cell infiltration.

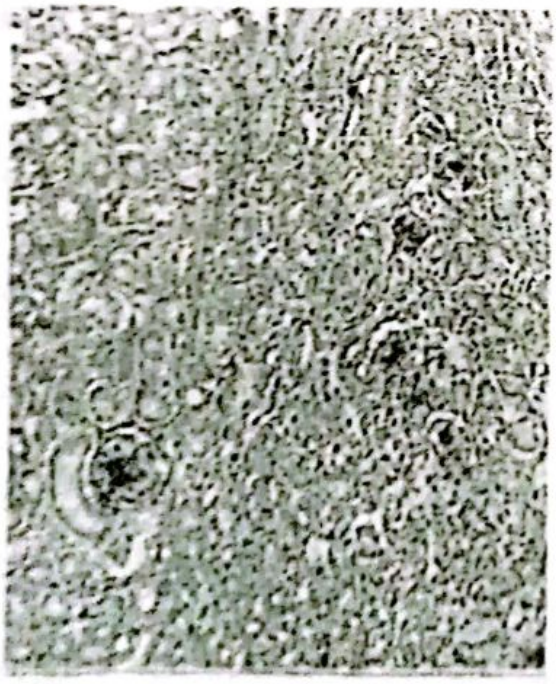


Fig. (6): Necrobiotic changes in the epithilium lining of proximal and distal convoluted tubules as well as cast cell formation with haemorrhage.

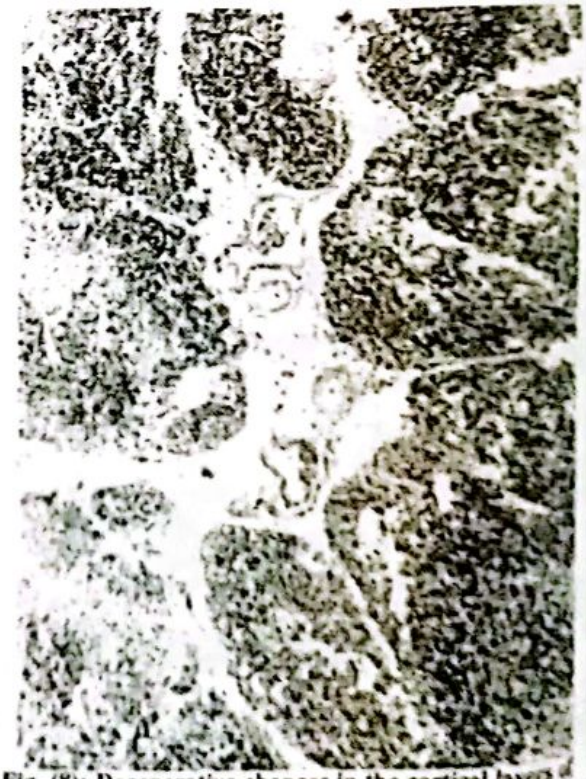


Fig. (8): Degenerative changes in the cortical layers of the thymus.

degeneration of hepatocytes as well as necrobiotic changes (Fig. 4). Congestion of central veins with mild infiltration with monocytes, vaculation in hepatocytes was seen which supposed to be fatty change.

All cases of examined lungs showed emphysema (Fig. 5) with congestion of pulmonary vessels and intra alveolar cappillaries as well as mononuclear cell infiltration were seen around alveolar septa (Gig. 5).

Necrobiotic shanges of the lining epithelium of the proximal and distal convoluted tubules in kipheys were noted in addition to cast cell formation and haemorrhages (Fig. 6). Focal degeneration and coagulative necrosis of myocardium were seen in one case, also focal areas of inflammatory cell infiltration between nuscle fiber is pronounced (Fig. 7). On the thymic tubule the cortical tissue occupy a narrow zone, degenerative changes in thymocytes nad epithilio reticular cells is advancad in relation to age (Fig. 8).

DISCUSSION

Analytical findinge of hen's serum revealed highly singificant elevatio in fluoride content (0.768 ppm) in comparison with control group (0.028 ppm). The elevation of fluoride in serum in addition to the skeletal changes consicered the main diagnostic tool for endemic fluprosis (Beddek, 198).

In domestic animals fluorine content in plasma was considered normal for the values under 0.3 ppm whereas animals with heavy fluorosis have more than 0.5 ppm; Greenowood et al., (1964). The analysis of feed stuff, water and air at arrea surrounding the supper phosphate factory revealed highar levels of fluoride, (Ibrahim, 1983) and (Seddek, 1988). Dur result revealed lower calcium levels in serum while phosphorus was elevated than control hens.

The activity of JGT in exposed hens was correlated to the pathological findings of degenerative changes of either liver (Fig. 4) or kidney (Fig. 6) ensure a release of such enzyme in serum. In the other hend the decreased activity could be referred to another factors, one of them could be put in mind that high fluorine level in serum.

Bone tissue had higher Ca%. Rich and Esinck (1981) reported extraordinary retention of calcium in patients with oestecporosis who had recieved 60 mg fluoride daily for several months. Tambers and Olsson (1970) suggested from the morphological and kinetic data that exposure to high levles of fluorides in the drinking water results in achronological sequences of a transient phase of hypermineralisation, followed by a supression of gastrointestinal absorption of Ca and a functional adaptation of the bne resorption with subsequent loss of bone mineral. The Ca/p molar ratios of bone minerals had been found to either remain unchanged (Zipkin et al., (1960); Singer et al., (1974) and McClure et al., (1958) of increased slightly (Bang, 1978) under the effect of flucrides. Out results indicated a decreased Ca/p ratio in hen's serum while calcium/phosphores ratio was increased in bone of affected hens (Table 6) than normal hens. The increased Ca/p ratio were generally due to increased Ca% rather than phosphorus depletion which appeared at the same level in affected and normal hens. Increased phosphorus levels in serum had been noticed by Seddek (1988) in fluoretic goats.

Dry matter and Ash% were determned and showed a highly significant increase of ash% either in relation to wet or dry base. The present data provide a support to the hyper mineralisation of bone indicating hyper calcification which could be ensured by increased Ca/e ratio. The results of Merkley (1981) indicated that the percentage of bone ash and fluprine content of ash followed a similar pattern in a commecial strain of which leghorn pullets.

The bone abnormalities which found in most casesindicate to a greater extent that fluoride stimulate bone formation through osteoplast activation this fact ensured by the presence of uncontroled growthes in the sternum. The bone lesions are similar to those describded by Seddek (1988) Grunder (1972). Shupe et al., (1963) in large animals. The fluorine content of bone which reached about 5000 ppm is considered responsible for the bone lesions. Rand nad Schmidt (1952)

considered more than 4000 ppm F a deleterious level of exposed animals. Spencer et al., (1971) in the other side suggested that 1800 ppm F is the normal of bone of cattle not suffering from fluorosis. Jones (1977) found F in affected cows bone levelss ragned from 3500-7100 ppm. Norberto et al., (1983) recorded that in birds recieving added fluorides one contains 3-10 fold qreater than normal (814 and 5500ppm), respectively.

The two fold increase in T3 T4 in addition to the decrease of iodine levels in serum indicates the stimulation of thyroid gland either directly or indirectly through increased fluorine levels in serum. Phillips (1936) have shwon that fluorine alone does not lower the basal metaboic rate but that it enhances the toxicity of hyperthyroidism induced by feeding desicated thyroid.Hatfield et al (1942) found a very marked decrease in the thyteid gland fresh weights and in the dry weights as fluorine increased proportionally with fluoride adminatration.

The results of the haematological picture of investigated hens resvealed a significant increase in RBCs count, haemoglobin content and FCV values. Decreased total leucocytic count was detected (Tables 2 and 3). These results are in agreement with that obtained by Ibrahim (1992) who revealed similar haematological effect on fish exposed to some industerial pollutant included flouride.

Total serum proteins and its fractions are affected by various environmental factors (Cles, 1986) levels of total serum proteins in affacted hens in this study were evident to show a significant decrease in comparison with the control group of hens (Table 4). This could be attibuted to the inhibitory effect fo florourine on protein synthesis which explored by Vesco and Colambo (1970). Albumin, globulin ration has been used to aid in interpretation of total protein values. The ratio will remain normal if both fractions are uniformly altered and be abnormal if an alteration in one fraction predominates (Robert al Keith, 1988). The results of the present study indicated a significant decrease in albumin level in investigated hens in comparish with the control group. The decreased amounts of serum proteins and increased fractions of gamma globul considered the most diagnostic tool envolvement of both liver and kidney dise specially chronic cases Schalm (1979).

Excessive loss of albumin a condision prev primarily as hypo albumineamia is present in c of hephrotic syndrome, glomeriolonephritis a result of some toxic substances. Albumin i major liver-synthesized protein, dur to liv affection the primary reflection to the ca hypoalbumineamia (Moragg. 1991). The record degenerative changes in the kidney and liv reported by Blood and Henderson (1983), severe cases of fluorosis support the prese finding.

Analytical investigation of T3, Ta 4 and iodi levels in blood serum revealed a highly signific elevation than normal (Table 5).

Triiodothyronine (3,5,3-Triiodo-1-Thyronine, T and thvroxine (T4) are the two active thyro hormones found in the blood strean Approximately 20% of circulating T3 is derive from direct synthesis and secretion by the thyro gland, while 80% is produced by deicdination T4 in peripheral tissue)Larson, 1972) and Sutige 1974).

The determination of total serum T3 in parameter used in the differentiation and clinic diagnosis of thyroid disease, particularl hyperthyrcidism Eastman et al., (1975) in mos hyperthyroid patints, both serum T3 and T4 ar elevated. Serum T5 levels are also an excelle indicator of the ability of the thyroid to respond t both stimulatory and suppressive tests Berger an Quinn (1976).

The histopathological changes had been ensure by Seddek (1988) in goats. It is obvious tha fluorine behave in the same way its effect in a biological processes inducing a similar pictur Both acute and chronic fluorine intoxications ar known to cause lesions in the kidneys of rats. I acute cases Ogilivvle (1953) found degenerativ changes in the collecting tubules with oedema i the renal papillae. Jankauskas (1974) foun necrosis in the tubules specially the convolute portions of the tubules. The emphysemato

lobules in lungs due to the irritant gases (HF) and heart muscle degeneration and necrosis had been recorded by Seddek (1988).

I would like to express my gratitued to professor Dr. M. G. Aggour for his most willing helps.

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