قسم : طب الحيوان وأمراض الدواجن .
كلية : الطب البيطرى - جامعة أسيوط .
رئيس القسم : أ.د . ابراهيم سكر .

صورة الخلايا الدموية الحمراء والبيضاء في حالات التسمم التجريبي تحت الحاد بالفلورين في الاغنام

أحمد عامر ، مجدى حافظ

أظهرت الدراسة أن العدد الكلي للكريات الدم الحمراء لم يتأثر معنويا بالتسمم التجريبي تحت الحاد بمركب فلوريدات الصوديوم (٢ مجم لكل كجم وزن حى لمدة ٢١ يوما) أو عند علاج مثل هذه الحالات بمركبات الكالسيوم أو الألومنيوم في حين كانت هناك اختلافات معنوية في تركيز هيموجلوبين الدم ونسبة الخلايا المصمتة بين المجموعات تحت الدراسة كما وأن العدد الكلي للكريات الدموية البيضاء والتصنيف النوعي لهذه الخلايا قد تأثر تماما سواء تحت تأثير التسمم أو عند العلاج بمركبات الكالسيوم أو الألومنيوم .

Dept. of Medicine & Poultry Diseases,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. I.H. Sokkar.

RED AND WHITE BLOOD CELLS PICTURE FOLLOWING EXPERIMENTAL SHORT TERM SUBACUTE OVINE FLUOROSIS

(With 6 Tables)

By
A.A. AMER and A.M. HAFEZ

(Received at 6/4/1983)

SUMMARY

Red and white blood cells picture was studied in sheep orally dosed daily with 2 mg/kg. Bwt. sodium fluoride and following calcium and aluminum treatment of such cases. Total red blood cell count was insignificantly affected while significant ($P < 0.05$) changes were recorded for haemoglobin content and packed cell volume. Total white blood cells count was significantly varied. The differential leucocytic count was altered following toxication with sodium fluoride and after calcium therapy (calphon) of toxic cases.

INTRODUCTION

HILLMAN, BOLENBAUGH and CONVERY (1978) observed the development of anaemia in cattle affected with industrial fluorosis. This was proved by low red and white blood cells count, haemoglobin content and haematocrit value in fluorotic herds. In another study (1979) the same authers proved that leucocytosis and eosinophilia with anaemia appeared in dairy herds with varying severity of dental and bone fluortic lesions. Eosinophilia was interpretted by HOOGSTRATON et al. (1965) to be an early indication of fluoride toxicity in bovines. Aplastic or hypoplastic anaemia, in the openion of VON OETTINGEN (1958), is the resultant of more or less severe inhibition of the activity of bone marrow in fluorotic cases. Relative lymphocytosis was also established by the same auther in such conditions. Oral dosing of sheep with 15 mg/kg. Bwt. sodium fluoride was followed three days later by an increased erythrocytic sedimentation rate and reduction in haemoglobin content and total erythrocytic count (TIWARY, SINGH, JHA and SINHA 1975).

It is the aim of the present study to trace the possible changes in red and white blood cells picture following short term sodium fluoride toxicity in sheep. The study was extended to estimate the value of treatment of such cases, with calcium and aluminum preparations on their blood cellular elements.

MATERIAL and METHODS

Full scheme of the experiment is illustrated in table (1). Anti-coagulated blood samples were collected from each animal four hours following morning feeding. Disodium salt of ethylenediamine tetraacetic acid was the anticoagulant of choice (1 mg/ml. blood). Haemotological examination of collected blood samples included total erythrocytic count (T.E.C.-$x10^6$/uL), haemoglobin content (Hb-gm/DL), packed cell volume (P.C.V.%), total leucocytic count (T.L.C.-$x10^3$/uL) and differential leucocytic count (%). Standard haematological methods were applied (COLES, 1980). Statistical analysis of obtained results was conducted on the basis stated by SNEDECOR (1956).

RESULTS

Red blood cell picture:

From table (2) it appeared that experimental subacute short term fluorosis in sheep resulted in an increased red blood cells count in the toxic group at the first (13.1x10 /ul) and second week (13.5x10 /ul) post-dosing which was followed by rather lowered red blood cell count at the third week post dosing. Recorded level at that time was 9.5x10 /ul. Transient lowered (7.2x10 /ul) red blood cell count was evident in the calcium treated group at the first week post dosing that was followed by gradual increase in the total red cell count at the 2nd (10.4x10 /ul) and 3rd weeks (10.7x10 /ul) post dosing. It is important to indicate that all post dosing levels in this group were always below their respective predosing levels (Table 2). Total red blood cells count in aluminum treated group behaved irregularily. Either the period of sampling or various treatment insignificantly affected total red blood cell count (Table 2).

A general tendancy for decreased haemoglobin content was evident at the second week post dosing in but all experimental groups when compared with predosing (Table 3). By the end of the experimental period, recorded haemoglobin content was somewhat resembling the predosing levels (Table 3) in the toxic and calcium treated groups. In aluminum treated group the level of haemoglobin at the end of the experiment was decreased by about 25% of the predosing level. Various used treatments and time of sampling significantly ($P/\_ 0.05$) affected haemoglobin content.

In spite of that the packed cell volume in all either experimental or control groups behaved variably during the whole course of the experiment (Table 4), yet the overall mean was higher in the toxic (38.85%) and aluminum treated (39.25%) group and lower in the calcium treated (34.93%) groups than the control animals (36.00%). Variations in packed cell volume between experimental groups or due to time of sampling were significant ($P/\_ 0.05$).

White blood cell picture:

Subacute short term fluorosis (group I) resulted in gradual leucopenia till the 2nd week post-dosing and then was followed by slight increased total leucocytic count at the end of the third week post dosing (Table 5). At that time recorded levels were however lower ($7.6x10^3$-/ul) than that the predosing level ($8.4x10^3$/ul). Treatment with calcium preparations induced significant leucopenia which lasted till the end of the experiment (Table 5). Aluminum treatment, on the contrary, insignificantly affected the total leucocytic count (Table 5). The overall mean of total leucocytic count, at the end of experiment was significantly ($P/\_ 0.05$) higher ($9.98x10^3$-/ul) in the control group than in the other three groups (7.40, 7.65 and $9.68x10^3$/ul respectively). The most lowered figures for those last groups was for the toxic one ($7.4x10^3$/ul).

Analysis of data for differential leucocytic count revealed that general neutrophilia accompanied with lymphopenia was characteristic for calcium treated group. The reverse was true for the first toxic group. Eosinophilia was rather evident at the end of the first and second weeks post post dosing in both toxic and calcium treated groups (Table 6). The other groups have had normal eosinophils percentages.

DISCUSSION

Analysis of data presented in tables (2, 3 & 4) revealed that the overall mean, of total red blood cell count, haemoglobin content and packed cell volume, intoxic group was always higher than that of control animals. On comparing data of this group at the end of the experiment with their respective predosing levels, it was noticed that figures for total red cells count

and haemoglobin content were generally lowered. Packed cell volume behaved a reverse character (Table 4).

Many authors (HILLMAN et al. 1979; SODEMAN, 1961 and VAN OETTINGEN, 1958) recorded that high levels of fluoridation of water or industrial intoxication produce anaemia. In an experimental study, TIWARY et al. (1975) observed that oral dosing of sheep with 15 mg/kg B.wt. sedium fluoride was followed three days later by an increase in erythrocytic sedimentation rate and reduction in total red blood cells and haemoglobin content. This high dose induced profuse salivation, lacrymation, dyspnoea, general hypraemia and gastroenteritis in experimental animals. The disagreement of the present data with those reported by TIWARY et al. (1975) could be attributed to the higher dose of sodium fluoride used by the later. Non of the clinical signs reported by TIWARY et al. (1975) were recorded by using sodium fluoride dose of 2 mg/kg B. wt. .

Treatment of toxicated sheep with either calcium or aluminum preparations for 21 days induced lowered total red blood cell count, haemoglobin content and packed cell volume, at the end of experimental period, if compared with predosing levels. Packed cell volume in aluminum treated group was exceptional.

From Tables (2, 3 & 4) one can safely said that daily oral dosing of aluminum mixture induced rather normal red blood cells picture. Although at the end of experimental period, the total red blood cells count in calcium treated group-amounted (10.78x10 /ul) that of control one (9.40x10 /ul), yet haemoglobin content and packed cell volume was higher in the second than the first group (11.23 gm/dl., 36%, 9.75 gm/dl and 34.93% respectively).

It appeared from (Table 5) that daily oral dosing of sheep with sodium fluoride (2 mg/kg Bwt.) for 21 days resulted in significant blood leucopenia ($7.4x10^3$/ul) when compared with control group ($9.98x10^3$/ul). Daily subcut. injection of calphon (Bayer)-30 ml for each animal failed to correct his leucopenia while daily oral dosing with aluminum mixture succeeded in prevention of leucopenia in toxic sheep (Table 5).

With regard to differential leucocytic count, it was recorded that toxication with sodium fluoride was accompanied by relative neutropenic and lymphocytosis changes while treatment with calcium salts (Calphon) induce reverse action. Meanwhile eosinophilia appeared in both groups by the end of the second week. (Table 6). A relative lymphocytosis was stated by VAN OETTINGEN (1958) as accompanies fluoride toxicity. HOOGSTRATON et al. (1965) concluded that eosinophilia could be used as an early indicator for fluoride toxicity in bovines. HILLMAN et al. (1979) pointed out that cows with various severity of dental and bone fluoritic lesions have had leucocytosis and eosinophilia.

Daily oral dosing with aluminum mixture was proved to be the best preventing agent against the abnormal leucocytic changes since the leucocytic picture (total and relative) invariably differed from that in control group (Table 5 & 6). In another words, it counteracts toxic activity of sodium fluoride, a fact which was assessed by ABD-EL ALL et al. (1981) where rather normal blood serum fluorine level was recorded in aluminum treated group when compared with control or toxic group.

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Table (1): Programme of Experimental Study.

Groups	Animals number	Method of dosage	Time and types of samples
I	4	given 2 mg/kg B.wt. from sodium fluoride daily for each animals, per os using stomach tube.	The experiment extended one month, Blood samples were collected weekly pre and post-dosing.
II	3	given the same dose of sodium fluoride plus calcium therapy in the form of calphom "24% calcium salts" S/C, 30 ml for each one.	
III	3	given the same dose of NaF plus aluminum mixture* in a dose of 30 mg. of aluminum to whole B.wt. per os.	
IV	3	given normal ration without administration of any dosage of drugs.	

* Aluminum mixture is consisted of equal parts of aluminum sulphate and aluminum oxide.

Table (2): Total Red Blood Cells Count ($x10^6$/ul) in Experimental Animals.

Group	Pre-exp.	Time of sampling post-dosing			Ex	$\bar{X}$
		1st.week	2nd.week	3rd.week		
I	10.1±2.1	13.1±3.3	13.5±1.9	9.5±4.5	46.2	11.55
II	14.8±0.9	7.2±2.4	10.4±3.4	10.7±5.5	43.1	10.78
III	10.8±2.5	9.4±1.3	10.5±3.4	7.6±4.9	38.3	9.58
IV	11.1±1.9	10.6±2.3	6.7±0.9	9.2±2.1	37.6	9.40
Ex	46.80	40.30	41.10	37.00	165.20	
$\bar{X}$	11.70	10.08	10.28	9.25		

Table (3): Blood Haemoglobin Content (Gm/DL) in Experimental Animals.

Group	Pre-exp.	Time of sampling post-dosing			Ex	$\bar{X}$
		1st.week	2nd.week	3rd.week		
I	11.8±2.0	11.8±1.6	10.3±1.8	11.6±1.0	45.50	11.38
II	9.9±1.8	9.8±3.1	9.5±1.2	9.8±3.5	39.00	9.75
III	13.5±2.0	12.8±4.4	10.6±3.0	9.9±1.5	46.80	11.70
IV	12.0±1.1	12.0±2.7	11.0±0.4	9.9±0.9	44.90	11.23
Ex	47.20	46.40	41.40	41.20	176.20	
$\bar{X}$	11.80	11.60	10.35	10.30		

ANOVA of Total Red Blood Cells Count.

S.V	D.F	SS	M.S	F	$t_{0.05}$	L.S.D
Period	3	12.445	4.1483	0.792	N.S	
Group	3	12.485	4.1616	1.258	N.S	
Error	9	47.140	5.2377			
Total	15	72.07				

N.S = Non significant at 0.05.

ANOVA of Haemoglobin Content

S.V	D.F	SS	M.S	F	$t_{0.05}$	L.S.D.
Period	3	7.6475	2.5492	3.87*	2.26	1.295
Group	3	8.9725	2.9908	4.54*		
Error	9	5.9175	0.6575			
Total	15	22.5375				

* Significant at 0.05.

Table (4): Packed Cell Volume (P.C.V %) of Experimental Animals.

Group	Pre-exp.	Time of sampling post-dosing			Ex	$\bar{X}$
		1st.week	2nd.week	3rd.week		
I	37.5±3.3	35.3±2.6	40.3±8.9	42.3±4.3	155.40	38.85
II	35.7±7.6	33.0±5.2	33.0±5.0	38.0±3.6	139.70	34.93
III	36.7±1.0	37.0±1.0	42.0±3.5	41.3±2.1	157.00	39.25
IV	34.3±3.2	32.3±2.1	38.7±6.7	38.7±3.8	144.00	36.00
Ex	144.20	137.60	154.00	160.30	596.10	
$\bar{X}$	36.05	34.40	38.50	40.08		

Table (5): Total White Blood Cell Count ($x10^3$/ul) of Experimental Animals.

Group	Pre-exp.	Time of sampling post-dosing			Ex	$\bar{X}$
		1st.week	2nd.week	3rd.week		
I	8.4±1.8	7.5±0.7	6.1±2.2	7.6±1.0	29.60	7.40
II	10.5±3.8	7.3±1.6	6.2±2.1	6.6±2.4	30.60	7.65
III	10.1±2.3	10.0±2.4	9.1±3.1	9.5±0.5	38.70	9.68
IV	10.6±2.2	10.3±1.9	9.3±0.4	9.7±4.6	39.90	9.98
Ex	39.60	35.10	30.70	33.40	138.80	
$\bar{X}$	9.90	8.78	7.68	8.35		

ANOVA of P.C.V. Values.

S.V	D.F	SS	M.S	F	$t_{0.05}$	L.S.D
Periods	3	76.4225	25.47416	9.5670*	2.26	2.607
Groups	3	54.1125	18.03750	6.7741*		
Error	9	23.965	2.6627			
Total	15	154.500				

* Significant at 0.05.

ANOVA of Total White Blood Cell Count

S.V	D.F	SS	M.S	F	$t_{0.05}$	L.S.D
Period	3	10.465	3.488	5.80*	2.26	1.237
Group	3	21.465	7.155	11.92*		
Error	9	5.400	0.600			
Total	15	37.330				

* Significant at 0.05

Table (6): Differential Leucocytic Count (%) of Experimental Animals.

Groups	Pre-dosing						Time post-dosing																	
							1st. week						2nd. week						3rd. week					
	Ba	S	L	E	B	M	Ba	S	L	E	B	M	Ba	S	L	E	B	M	Ba	S	L	E	B	M
I	-	30.50 ± 9.46	69.50 ± 8.10	4.00 ± 1.70	1.30 ± 0.70	0.8 ±	2.50 ± 2.80	32.80 ± 11.10	55.00 ± 7.80	7.50 ± 4.40	0.3	2.00 ± 0.80	-	27.80 ± 2.22	62.70 ± 3.80	7.50 ± 2.20	-	2.00 ± 0.60	1.50 ± 4.40	32.00 ± 8.50	62.80 ± 6.02	3.00 ± 1.70	-	0.80 ±
II	-	31.30 ± 9.60	63.30 ± 10.10	4.30 ± 1.75	-	0.8	-	51.00 ± 11.80	39.00 ± 13.50	7.00 ± 2.00	-	3.00	4.70 ± 3.20	41.70 ± 10.20	43.30 ± 8.60	8.30 ± 3.21	0.3	1.70 ± 0.70	5.50 ± 0.70	67.30 ± 15.01	26.70 ± 13.78	0.30	0.3	1.00
III	1.7 ± 0.57	29.70 ± 4.04	61.30 ± 3.50	5.70 ± 4.04	0.30	1.30	4.00 ± 9.50	32.00 ± 2.30	57.30 ± 1.50	3.30 ± 1.50	-	3.30 ± 0.71	4.50 ± 2.10	35.70 ± 4.50	53.00 ± 2.50	7.00 ±	-	1.00	1.30	29.00 ± 7.00	64.30 ± 7.02	4.00	-	1.30
IV	3.00 ± 1.70	42.00 ± 13.00	48.30 ± 8.60	4.00 ± 3.00	0.70	2.30	7.00 ± 3.00	32.70 ± 11.10	53.60 ± 15.50	5.00 ± 1.70	-	1.70 ± 0.60	3.70 ± 2.50	36.70 ± 8.60	51.70 ± 10.40	6.30 ± 2.10	0.7	1.30 ± 0.57	4.00 ± 1.50	31.30 ± 6.40	58.00 ± 7.20	4.30 ± 1.20	-	2.30 ± 1.15

Ba = Band S = Segmented L = Lymphocyte E = Eosinophil B = Basophil M = Monocyte