قسم طب الحيوان
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نمط التحليل الكهربي لبروتينات مصل الدم في حالات تنكرز الكبد في الأغنام

علي السباعي ، أحمد عامر

أجريت الدراسة على عدد ٣٥ رأسا من الأغنام الاوسيمي منها ٢٥ رأسا مصابة بمرض تنكـرز الكبد بينما استخدمت عشرة رؤوس من الأغنام السليمة صحيا بغرض المقارنة مع المجمـوعـة المريضة ٠ أظهر التحليل الكهربي لبروتينات مصل الدم في الأغنام السليمة أن نسبة البروتين الكلي ، الالبيومين ، الالفا ، والبيتا ، والجاما جلوبيلين على النحو التالي ٧٤ر٥٥ جم / لتر ٦٣ر٥٥٪ ، ٩ر٦٤٪ ، ٦ر٦١٪ ، ٩ر٥٨٪ ، ١٨ر٥٨٪ على على التوالي مقارنة بنتـائـج المقابلة في الأغنام المريضة والتي كانت ٥٠ر٧٤ جم /لتر ، ٣٧ر٨١٪ ، ١٥ر٠٢٪ ، ٢٣ر٣٤٪ ٢٣ر٨٢٪ ، ٢٣ر٨٢٪ على التوالي كذلك ٠

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ELECTROPHORETIC PATTERN OF OVINE BLOOD SERUM PROTEINS IN CASES OF NECROTIC LIVER DISEASE

(With 2 Tables & 4 Figs.)

By
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SUMMARY

This study was carried out on 35 native breed Osimi sheep, Ten animals were aparantly healthy and kept as control. Twenty Five sheep were disease and showed a signs of liver illness in the form of depression aneroxia, diarrhea or constipation and in late stage recumbency and death. The mean values for serum total protein, Albumen, α_1, α_2 B and -globulin in healthy sheep were 74.55 g/l 55.63%, 9.61%, 9.58% and 18.58% respectively while in diseased sheep the values were 50.47 g/l, 37.81%, 15.02% 23.34%, 23.82% and 23.82 respectively.

INTRODUCTION

Many clinical laboratories have attemped the use of the changes observed in the quantitave and qualitative composition of the plasma protein for spesific diagnosis of the disease (CORNELIUS and KANEKI, 1960). Alleration that have been observed, have only served to demonstrate the state of the subject under study of particular time (INFRAN, 1967).

The abnormalities that are observed in plasma proteins can be grouped into number of patterns which characteize entire group of pathological states. These conditions are acute inflamation, chronic inflamatory and proliferative processes liver and biliary disorders, nephrotic syndrome and carcinomas.

Since the liver plays a central role in both anabolism and catabolism of plasma proteins, its not unreasonable to expect that plasma proteins analysis would be usuful in detecting liver injury (SCHALM, 1975). Unfortunately, protein fraction is of tittle value in detecting the degree of the lost function, or the establishing presence of parenchymal damage (SCHALM, 1970).

Liver damage does produce charactestic changes in plasma protein, however these changes appear late in the processe and may be more prognostic than diagnostic (SCHALM, 1975). Hypoprotenaemia commonly observed in hepatic diseases parasitic infestion, kidney disease and febrile diseases causes breakdown of endogenae protein (CORNELIUS, 1960). Generally the most striking changes in disease is observable as decrease in the albumin fraction. The decrease may be the result of an inhibition in the synthesis or more rapid catabolism or it may be due to an increase in the concentration of globulin (HERZ and HOD, 1969, LARSON & KENDALL, 1957). Moreover changes in γ -globulin reflects the response of reticuloendothilial system of antigens and these appear to be correlation between the concentration of γ -globulin and antiboey titer. The case is so in most infections with perhaps-the exception of those of viral etiology (DIMOPOULLOS, 1961).

Necrotic hepatitis (Black disease) is mainly a disease of sheep, but in some areas it is also of significance in cattle steers. The disease is usually caused by Cl.novyi and precipitated by invasion of liver by immature liver fluke (BAGADI and SEWELL, 1974).

Due to the great destructive effect of the disease on the liver cell, attention in the present investigation was paid to study the extent of changes in total serum proteins, and serum proteins electrophoresis in spontanous cases of necrotic liver disease in sheep.

MATERIAL and METHODS

In this study 35 native - breed Osimi sheep of both sexes were included. The animals were belonging to El-Hawatka station for animal production.

Twenty-Five sheep showed the signs of illness in the form of depression, fever, anorexia in some cases recumbency while ten apparently healthy sheep were kept as a control.

Blood samples were collected from each animal by means of jugular veinpuncture in strile dry and clean centrifuge tube. Serum was seperated by the ordinary method of veterinary hematology serum total proteins was estimated using test kits (BioMerux-France) and measured by Bye-unicum spectrophotemeter 8800 at wave length 546 nm.

Fractionation of serum proteins was carried out using 0.8 ul of serum by Hamlton syringe and agarose film. The film was then processed fro approximately 35 minutes using 95 ml of universal barbital buffer in each chamber of cells. At completion of the electrophoretic seperation the film was placed in 200 ml of amido-black 10 B working solution for 15 minutes removed from the stain solution and then rinsed in 20 ml of 5% acetic acid clearing solution using magentic stirres operating for 30 seconds.

The film was then compeletly dried for 20 minutes then allowed cool at room temperature, then washed in 5% acetic acid clearing solution to clear the excess stain proior to drying for one minute with agitation. It is then transfered to a second stirr stain dish contain 5% acetic acid solution, rinsed again for one minute, untile the excess stain is removed and dried for 15 minutes. Densitometry of the stained film was performed using DCD - 16 digital computing densitometer Gelman instrument company fitted with 520 nm interfrence filter. The values of optical density were plotted automatically by the aid of the instrument. The results were expressed by tacking as zero the migeration of gamina globulin and as 100 that of the albiumin.

RESULTS

Clinical observation of diseased sheep revealed anorexia, depresion, fever and intermittent diarrhoea and constipation. In some cases the animals unwill to move and recumbant. Such clinical manifectations were concised to liver affection and previausly diagnosed as necrotic. Liver disease (black disease) associated with cl. novyi infection and accompained with migration of immature forms of liver fluke (EL-SEBAIE, et al. 1987).

Results of total serum proteins and protein electrophoresis of healthy and diseased sheep were presented in table (1 & 2) and figures (1, 2, 3 & 4).

Mean values of serum total protein, Albumin, α -B and γ -globulin in healthy sheep were 74.55 g/l 55.63%, 9.69%, 6.61%, 9.581% and 18.58% respectively, while the Albumin/globulin

ratio was 1.256. These values for disease sheep were 50.47 g/l 73.81%, 15.02%, 23.34%, 23.82% respectively and the A/G ratio was 0.61.

DISCUSSION

In spite of the great range of total serum proteins mentioned after different authers, it is clear here to mention that the mean value of serum proteins in healthy individual lied with the range and accepted with the results published after CORNELLIUS (1960), CORNELLUS and KANEKO (1968), DOXEY (1978) and HASSAAN, et al. (1984).

The results of serum protein electrophoresis are in close agreement with those reported by CORNELLUS (1960), and HASSAAN, et al. (1984). On the other hand our results disagreed with the findings of SCHALM (1970). Conserning the Alpha and Beta globulin ratio-, such varitions could be due to the methods adopted.

Screening the data of serum protein in diseased sheep, there is a strong evidence of significant hypoproteinaemia (50.47 g/l) in contrast to mean values of healthy group (47.55 g/l). Such drop in the mean value of total serum protein could be attributed to the faliure of the liver for protein sunthesis under the influence fo cl. infection or due to excessive liver cell damage during the migeration of liver fluke (CORNELLUS, 1960; CORNELLUS and KANEKO, 1968 and SCHALM, 1970). Hypoalbumenaemia (37.81%) is the constant finding in individuals showing the signs of necrotic liver disease. This finding is accepted with CORNELLUS (1960). The author refered such atteration in blood serum albumin to retarded protein synthesis from such common clinical hepatic necrosis and cirrohsis due to spesific cause.

Mean value of gamma globulin is markedly increased (23.83%) in diseased group. It is wellknown that increase in γ -globulin value refects the response of reticuloendothilial system to the causitive antigen (DIMOPOULLOS, 1961). Due to the marked decrease in allumin concentration and marked increase in value of serum globulin in diseased sheep, the ratio is consequently decreased of compared with healthy cones.

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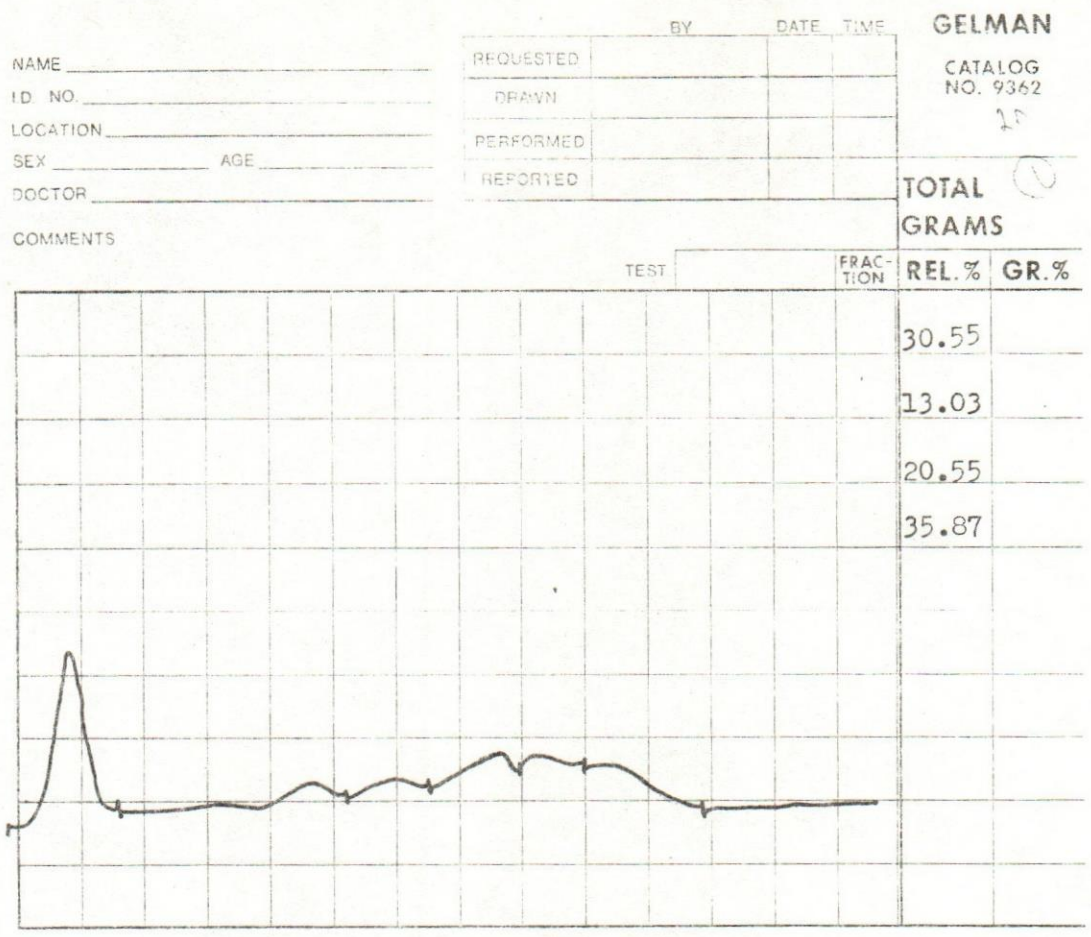
Table (1)
Serum total protein and serum protein electrophoresis in healthy sheep

No	T. Protein g/l	Alb.	α_1	α_2	B_1	B_2	γ - globulin	Globulin	A/G
1	75.31	56.70	9.60	8.4	6.60	18.70		43.34	1.30
2	71.46	58.30	8.60	9.20	6.50	17.		41.44	1.40
3	78.07	55.10	11.40	5.30	13.49	19.71		49.9	1.10
4	70.32	57.40	12.30	3.71	9.79	16.80		42.60	1.34
5	73.05	50.90	9.90	9.70	9.90	19.60		49.1	1.03
6	74.60	51.94	8.60	6.80	13.90	18.71		48.01	1.08
7	75.30	55.60	7.35	3.90	15.21	17.94		44.4	1.25
8	76.10	58.30	12.4	2.4	10.30	16.60		41.7	1.39
9	74.50	53.79	7.73	8.30	5.35	19.83		41.2	1.30
10	77.80	58.30	8.00	8.4	4.7	20.60		42.3	1.378
x	74.551	55.633	9.648	6.611	9.581	18.58		44.39	1.256
S.D	±2.487	±2.694	1.825	2.59	3.737	1.36		3.34	0.137

Table (2)
Serum total protein and protein electrophoresis in sheep with necrotic liver disease

	Total protein	Alb. %	α-1 %	α-2 %	B_1 %	B_2 %	γ %	Total Glob. %	A/G %
1	57.20	31.78	6.17		39.46		22.39	68.02	0.46
2	29.30	28.55	15.18		31.35		24.92	71.45	0.39
3	50.73	34.06	11.30		26.04		28.60	65.94	0.51
4	33.82	29.50	13.10		33.39		24.01	70.50	0.40
5	41.80	34.63	16.34		27.33		21.70	60.37	0.57
6	59.85	40.9	20.40		19.00		19.70	59.10	0.69
7	47.69	31.01	9.07		23.62		36.30	68.99	0.44
8	59.28	34.52	12.22		23.07		30.19	65.48	0.52
9	58.52	31.94	21.62		26.63		19.81	68.06	0.46
10	53.90	31.94	18.00		23.59		26.44	65.61	0.52
11	48.60	40.01	17.34		24.69		17.96	59.99	0.66
12	49.03	30.55	13.03		20.55		35.87	69.97	0.43
13	48.71	35.76	14.20		22.74		27.30	64.24	0.55
14	44.30	44.10	14.10		18.73		23.07	55.90	0.78
15	53.10	41.70	11.29		10.14		26.87	58.30	0.71
16	56.7	38.90	23.31		16.38		21.51	61.18	0.63
17	57.6	44.70	9.14		19.10		27.06	55.30	0.80
18	38.90	42.30	17.22		18.70		21.88	58.28	0.72
19	34.60	41.20	17.05		25.88		15.87	58.80	0.70
20	63.70	51.9	4.98		26.31		16.81	48.10	1.07
21	47.13	38.60	19.23		18.83		23.35	61.41	0.62
22	56.75	40.70	21.81		17.30		20.19	59.30	0.68
23	55.10	43.20	18.24		18.86		19.70	56.8	0.76
24	46.80	41.30	20.30		19.7		18.70	58.70	0.70
25	59.70	39.06	13.49		22.11		25.34	60.94	0.64
x	50.479	37.810	15.02		23.34		23.82	62.029	0.616
S.D	±9.50	5.683	4.80		5.45			2.40	0.155



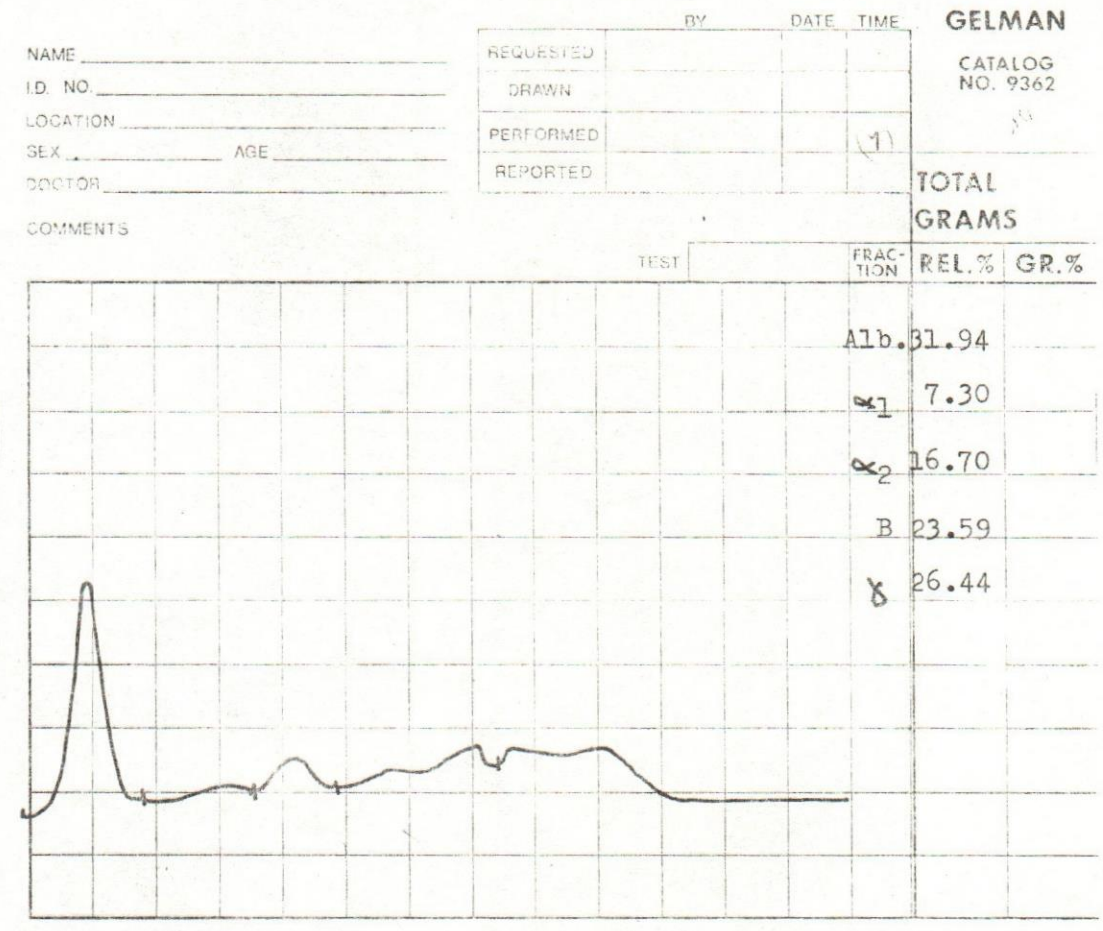


Fig. (1,2): Electrophoretic pattern of serum proteins in healthy sheep

NAME El-Hawatka

I.D. NO.

LOCATION

SEX AGE

DOCTOR Dept. of animal med.

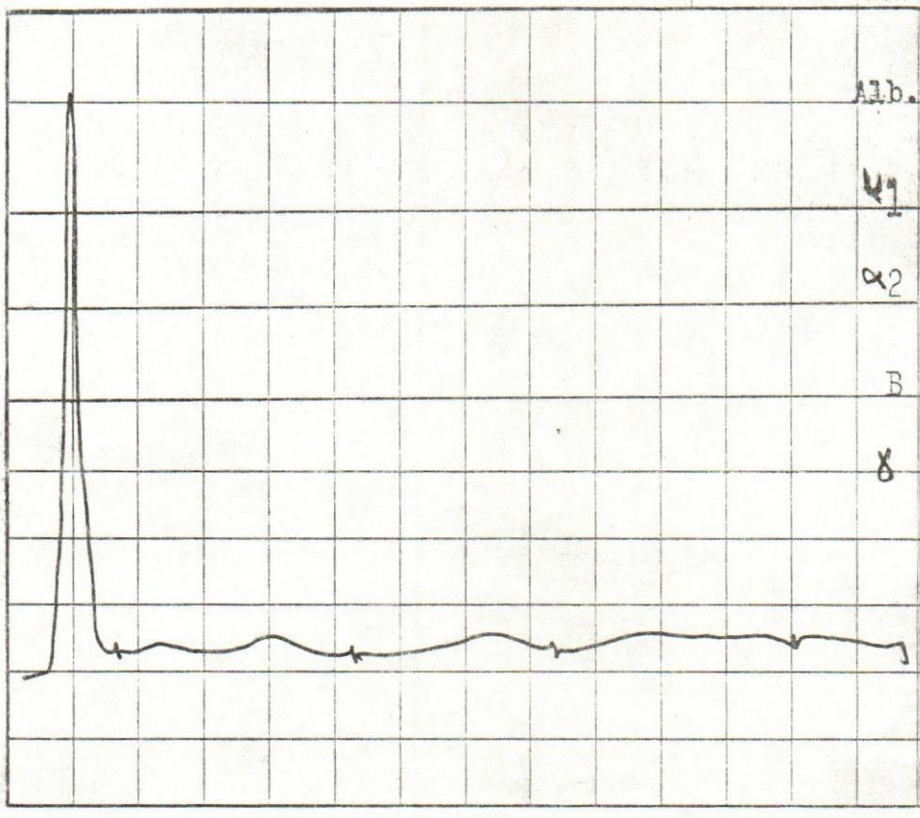
COMMENTS

REQUESTED			
DRAWN			
PERFORMED			
REPORTED			

CATALOG NO. 9362

TOTAL GRAMS

TEST	FRACTION	REL.%	GR.%
	Alb.	51.94	
	α1	8.60	
	α2	6.80	
	B	13.90	
	γ	18.94	



NAME El-Hawatka

I.D. NO.

LOCATION

SEX AGE

DOCTOR Dept. of animal Med.

COMMENTS

	BY	DATE	TIME
REQUESTED			
DRAWN			
PERFORMED			
REPORTED			

GELMAN

CATALOG NO. 9362

TOTAL GRAMS

TEST	FRACTION	REL.%	GR.%
	Alb.	56.70	
	α1	9.60	
	α2	2.4	
	B	6.60	
	γ	18.70	

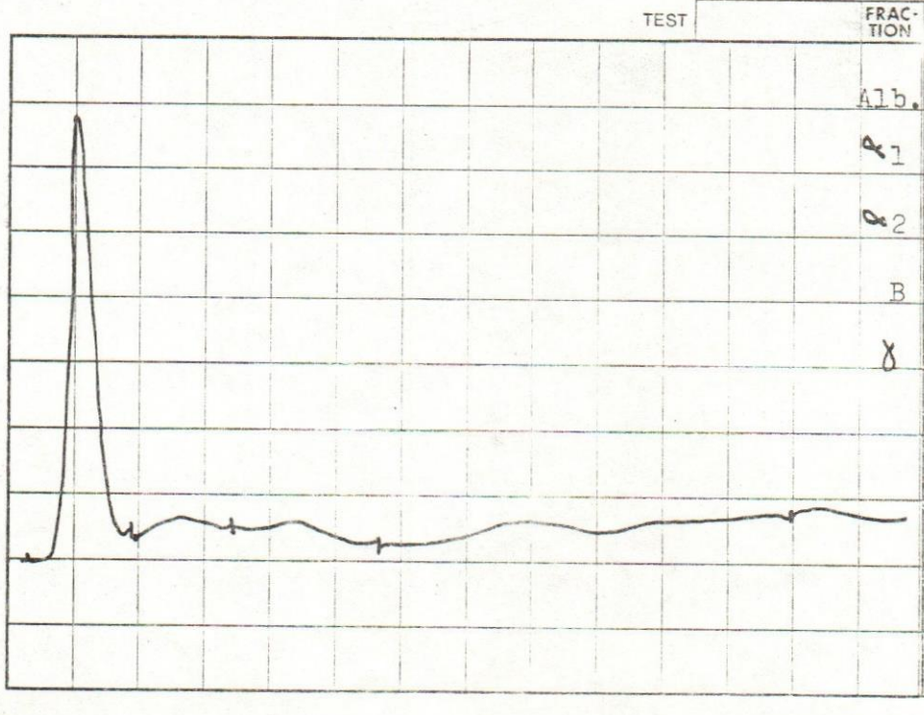


Fig. (3,4): Electrophoretic pattern of serum protein in sheep showed necrotic liver disease.