قســـــــــم : الأمراض الباطنة والمعدية ـ كلية الطب البيطرى ـ جامعة أسيوط.
رئيس القســـــم : أ.د / ابراهيم محمد حسن سكر.

الصورة الاكلينيكية والمعملية فى الجاموس والأبقار المحقونة تجريبيا بعترة ميكروب السل الكاذب السامة

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تم دراسة الأعراض الاكلينيكية والتغيرات التى تحدث فى الدم فى ١٠ عجول جاموس وخمس عجول بقرى بعد اصابتها تجريبيا بعترة سامة من ميكروب السل كاذب.

وقد لوحظ اختلاف طفيف بين الجنسين فى الأعراض الاكلينيكية . وقد لوحظ ايضـــــا نقص تدريجى فى عدد كرات الدم الحمراء والهيموجلوبين وخلايا الدم المصمتة فى كل مـــــن الجاموس والأبقار ولكن بالنسبة للأبقار كان هناك زيادة كرات الدم البيضاء اكثر منهـــا.فـــى الجاموس زادت نسبة GPT & GOT بعد الاصابة التجريبية فى كل من الابقار والجاموس ولكـــن بالنسبة لنتروجين اليوريا لوحظ بها نقص مبدئى طفيف ثم بعد ذلك زادت حتى وصلت اعلى من معدلها الطبيعى فى نهاية التجربة.

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CLINICAL AND LABORATORY INVESTIGATIONS IN CATTLE AND BUFFALOES EXPERIMENTALLY INFECTED WITH A TOXICNIC STRAIN OF C.PSEUDOTUBERCULOSIS

(With 4 Tables and 8 Figures)

BY

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SUMMARY

Clinical manifestations and blood changes were studied in 10 buffalo and 5 calves following experimental infection with toxigenic strain of C.pseudotuberculosis.

A slight species difference in the clinical picture between buffaloes and cattle was observed.

Cattle showed a tendency to leucocytosis than buffaloes. A gradual decrease in total erythrocytic count, haemoglobin and packed cell volume was observed in both buffaloes and cattle.

Serum GOT and GPT were increased after infection in both animal species, while blood urea nitrogen showed initial drop,then it began to rise until it reached a level above the normal.

INTRODUCTION

Cattle is known to be susceptible to infection with C.pseudotuberculosis; lesions were found in the skin, lymph vessels ans superficial and internal lymph nodes (NOCARD, 1888; VRYBURG, 1907; REYMOND, 1909; SCHIEGAL, 1913; SHEATHER, 1920; BULL, 1933; PURCHASE, 1944; LOVELL, 1959; RIISING and HESSELHOLT, 1973; ADDO and DENNIS, 1977). Likewise, buffaloes can be affected and the disease was found to be associated with lesious in the skin and regionol lymph nodes, oedema in the dewlap, abdomen, shoulder, side of the head and neck and sometimes infammation of the intestine and lungs (CARPANO, 1934; SOLIMAN et al., 1963; BARAKAT and EID, 1971; FOUAD et al., 1975). In other animal species, namely in sheep (CARRE and BIGOTEAU, 1908; BOQUET, 1942; ROBINSON, 1928; PURCHASE, 1944) and guinea pigs (PURCHASE, 1944) infection was occasionally accompained with haemoglobinuria and ictrus. A highly toxigenic strain of C.pseudotuberculosis was isolated from buffaloes and identified by BARAKAT (1979, 1980; 1981); this stain was experimentally used for infecting buffaloes and cattle by KHATER et al. (under publication), to whose material this investigation was carried out. The aim of the present work is to study the clinical manifestations and changes in the blood with special reference to kidney and liver function tests occuring under experimental infection with the strain.

MATERIAL and METHODS

Clinically healthy animals consisted of 10 buffalo-calves of an average age of 1.5 year and 5 calves of a native cattle breed 1-1.5 years old were used*; all animals were tuberculin tested and were proved to be free from both internal and blood parasites. Each animal was intradermally inoculated with 3 ml of serumized 24 hours broth culture of A Bu 77 strain of C.pseudotuberculosis. Inoculation was carried out at the circumference of a circle of 15 cm in diameter in the left shoulder region. On the right shoulder 0.2 ml of the same inoculum was intradermally inoculated at one site. Control animals were inoculated only with borth at the right shoulder. Infected animals were kept under observation, the temperature was recorded every 4 hours during the first day and then daily for the duration of the experiment which lasted 15 days.

For haematological and laboratory investigations,blood samples were obtained before infection and 1,2,4,7 and 15 day post-infection. These blood samples were examined for determination of total erythrocytic and leucocytic cell count, differential leucocytic count and packed cell volume (SCHALM, 1965), total haemoglobin (test-kits supplied by Boehringer, Mannheim, GMBH, W.Germany),serum level of glutamic-pyrovic and glutamic-oxalacetic

* : Two of these animals, from buffaloes and cattle, were kept as controls.

transaminases (GOT, GPT) (REITMAN and FRANKEL, 1957), serum glucose level (TINDER, 1969) using test-kits supplied by Bio-Merieux (France), serum total protein (WEICHSELBAUM, 1946) using test kits supplied by Boehringer, Mannheim, GMBH, W.Germany, serum urea level using test kits supplied by Bio-Merieux (France)and serum billirubin using test kits supplied by Boehringer Mannheim, GMBH, W.Germany.

RESULTS

Clinical manifestations:

In buffaloes, a hot painfull swelling appeared at to site of injection within 24 hours in all infected animals, this swelling reached its maximum size after 5 days. At this period, cutaneus fissuring of the skin covering the swollen area was observed. This local lesion was accompanied with oedematous swelling of the dewlap which was quite prominant in two animals.

In cattle,raised oedematous circular ridges with depressed center locating the site of inoculation was observed in the skin at the left shoulder in 3 of the infected animals as early as 12 hours postinfection. This area was sensitive to touch and hot,it subsided to a great extent at the second day. In the latter two animals, swelling of the skin was less remarkable.

The average temperature curve of all animals throughout the whole period of the experiment is illustrated in (Fig. 1).

In buffaloes, two waves of increased temperature were demonestrated, the first occured after 18 hours of infection and the second after 6 days followed by gradual drop until a normal level was reached. However, one animal which was scarified at the end of the experiment showed a rise of temperature during the last two days. In cattle, animals showed degree up to 41°C after 18 hours then the temperature fluctuated between 39 and 40°C to the 7th day after which it returned to normal.

Haematological findings:

Values of different parameters studied in the blood of experimental animals are shown in Tables I, II, II& IV. Blood of buffaloes revealed an average increase of white blood cells count within the first 24 hours which fluctuated but remained above the normal level determined before infection till the end of the experiment (Fig. 2). The differential cell count was characterized by intermittent lymphopenia and a relative neutrophilia accompanied with left shift. The average total erythrocytic count showed a gradual decrease which reached its maximum at the 7th day and returned nearly to its normal level in the animal sacrified at the end of the experiment (Fig. 3). The packed cell volume and haemoglobin concentration behaved in a rather similar manner (Fig. 4 & 5). It was also observed that in animals died during the experiment, often 6 and II days, a severe drop of erythrocytic count accompanied with low concentration of haemoglobin and decreased packed cell volume percent occured shortly before death. The sera of these animals revealed marked features of haemolysis.

Examination for enzyme activity of buffalo-serum revealed an increase in GOT which its maximum on the 4th day and remained above the normal level till the end of the experiment (Fig. 6) and an increase in GPT which reached its maximum after 24 hours and remained above the normal level till the end of the experiment (Fig. 7).

Determination of total serum bilirubin level revealed a stable results in different infected buffale calves, however, there was a tendency for increase which was marked in some animals, namely these sacrified at the 4th and 7th day of the experiment. Blood urea nitrogen showed an initial drop form the 1st day, then it began to rise until it reached a level above the normal in animals sacrified after the 7th and 15th day (Fig.8). Blood glucose level and serum protein remained within the physiological limits throughout the whole period of the experiment.

In cattle, the average white blood cell coûtn rapidly increased during the first 24 hours and remained higher than normal to the end of the experiment (Fig. 2). Differential leucocytic cell count revealed slight variation in the number of lymphocytes throughout the experimental period, while the number of neutrophils was increased during the first 4 days then decreased to below normal in the next II days (Table III). The average

total erythrocytic count, packed cell volume and haemoglobin concentration behaved nearly in the same manner, they decreased gradually from the 1st to the 7th day after infection then returned to normal at the end of the experiment. (Fig. 3, 4 & 5).

Serum activity of GOT in cattle was increased after 24 hours, fluctuated slightly around this level to the 7th day then gradually increased to reach its maximum level at the end of the experimental period (Fig. 6). A sharp increase of GPT occured 24 hours of infection, this increase remained also constant during the 2nd day. At the 4th day a slight decrease was observed and the activity remained stable to the 7th day followed by increase at the 15 day of experiment (Fig. 7). The concentration of the total serum bilirubin showed an initial drop in the last day, followed by a moderate increase during 2nd to 4th day then decreased again. A gradual drop of blood urea nitrogen occurred during the first 7 days of infection while a high level than normal was determined in an animal slaughtered at the 15th day of inoection (Fig. 8). Variations in the level of glucose and total serum protein throughout the experimental period were slight.

DISCUSSION

In the present study, clinical picture as a result of infection with the applied strain of C.pseudotuberculosis revealed slight species difference between buffalo and cattle. While oedema and swelling of the skin were marked in the first animal species, they were less extensive and localized to a narrow rim at sites of inoculation and subsided rapidely in cattle. In the latter, fissuring of the skin and swelling of the dewlap which were observed in some cases of buffaloes, were not found. In both animal species infection was associated with fever which was more noticeable during the first week of infection.

Examination of blood revealed that cattle has a more tendency to leucocytosis than buffaloes, a response which is known to be related to susceptiblity to infection. Higher count of white blood corpuscles in cattle was manily due to increased number of neutrophils circulating in the blood. As well, total erthrocytic count, packed cell volume and haemoglobin concentration were generally higher in cattle than buffalo collowing infection, however, the average values tended to decrease in both species after the first week postinfection.

Clinical manifestations of jaundice were clearly demonestrated in a buffalo-calf died II days postinfection. As well, high serum bilirubin level was demonestrated in buffalo calves from the second day and remained high in the majority of cases until death or sacrification. In cattle, the results were inconstant. The association of jaundice in infections with some strains of C.pseudotuberculosis has been known a long time ago and repeatedly referred to in the literature. CARRE and BIGOTEAU (1908) described a disease known as "Mal-Rouge" in France, while BOQUET (1912) reported a similar disease known as "El-rouch" in north Africa. Both diseases were characterized by icterus and haemoglobinuria and the authors believed to be caused by C.pseudotuberculosis. In addition, CARRE and BIGOTEAU (1908) found that intravascular haemolysis and haemoglobinuria or icterus occurred after sheep had been inoculated with cultures of virulent strain of C.pseudotuberculosis. ROBINSON (1928) reproduced an intensive haemolytic condition which he named "bacterial icterus" by inoculation of sheep with C.pseudotuberculosis recovered from abscesses. PURCHASE (1944) found that in sheep died after subcutaneous inoculation of broth culture, there was an intensive icterus and haemolysis. Three locally isolated strains of C.pseudotuberculosis from bovine, caprine and equine materials were compared by the auther by injecting them intracutaneously into guinea pigs. One of two animals infected with the bovine strain showed haemorrhage at the inoculation site, while the whole carcase was yellow in one animal and pink in the latter, accompanied with haemorrhages in the pericardium, lungs and adrenals. LOVELL and ZAKI (1966) suggested that C.pseudotuberculosis exotoxin appears to be identical or closely related to the substance producing haemolysis on blood agar, while CARNE and ONON (1978) declared that C.pseudotuberculosis is exotoxin is a sphingomyelinase which splits sphingomyelin of the cell membrane of erythrocytes into ceramide phosphate and choline.

Study of blood enzyme activity fo GOT and GPT showed an elevation of both of these two enzymes one day after infection indicating a disturbance of liver function. The latter was emphasized histopathologically by KHATER et al. (under publication, a,b) in the same animals. While blood urea nitrogen was constantly higher than normal in buffalo calves during the experimental period, such elevation was demonestrated only in one calf of

cattle group sacrified after 15 days. It can therefore be concluded that, under infection with C.pseudotuberculosis both buffalo and cattle showed similar effects with regard to the function of parynchymatous organs,mainly liver and kidney. This disturbance in function may be related to an exotoxin produced by the organism.

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Table (I)

Results of blood examination of calves before & after infection with C.ovis

Case No.	time	RBCs 16^6	WBCs 10^3	HB gm %	PCV %	GPT U/I	GOT U/I	Glucose mg%	Bilirubin gm%	T.P gm%	Urea mg %
1	Before	8.12	10.0	13.2	42	20	25.1	90	0.6	0.3	30
	24 hrs.	7.98	13.6	14.0	42	29	29.5	56	0.1	8.9	23
2	Before	6.31	9.4	14	42	20	22.0	80	0.2	11.9	28
	24 hrs.	6.12	14.4	11	38	27	29.0	74	0.1	8.6	16
	48 hrs.	5.98	12.6	14	42	31	36.5	38	0.5	6.2	18
3	Before	5.66	5.4	13.5	35	21.4	22.6	76	0.30	9.1	16.0
	24 hrs.	4.96	19.6	11.0	29	25.0	26.5	74	0.26	8.3	18.0
	48 hrs.	5.04	17.6	11.0	31	29.0	31.5	44	0.48	5.5	22.0
	4 Days	5.04	15.8	11.5	35	29.0	31.5	50	0.54	6.8	21.0
	7 Days	4.12	17.4	10.0	28	25.0	27.0	64	0.24	6.0	16.3
4	Before	8.52	9.6	14.0	46	21	21.4	86	0.60	8.9	30
	24 hrs.	8.13	14.2	13.5	40	28	27.0	50	0.26	5.2	13
	48 hrs.	6.86	17.8	14.0	42	29	29.5	34	0.30	5.6	17
	4 Days	6.75	14.6	13.0	40	27	33.5	53.5	0.48	6.9	17
5	Before	7.69	7.6	13.5	42	21.4	22.6	70	--	9.7	20.0
	24 hrs.	7.62	16.2	13.0	34	27	27.5	56	0.18	5.9	13.0
	48 hrs.	6.83	15.6	10.5	33	28	31.5	47	1.0	7.4	16.0
	4 Days	5.98	12.6	10.0	30	27	38.0	37.5	0.9	4.4	17.0
	7 Days	3.87	11.6	9.0	24	26	35.5	73.0	0.3	5.4	12.2
	15 Days	7.47	9.4	13.5	40	29.5	45.0	100.0	0.24	7.7	60.0

Table (II)

Results of blood examination in buffalo calves before and after infection with C.ovis

Case No.	Time	RBCs 10^3	WBCs 10^2	HB gm %	PCV %	GPT 1.U/I	GOT 1.U/I	glucose mg%	T.P gm%	Bilirubin mg%	Urea mg %
I	Before	7.87	10.4	13.5	50	21.4	23.9	80.0	8.02	0.3	21.0
Sacrif.	After 24 hrs.	4.43	12.6	9.0	25.0	32,0	34.0	67.0	5.94	0.1	17.0
9	Before	7.73	6.8	13.5	35	21.6	24.4	67.0	11.6	0.6	41
Sacrif.	After 24 hrs.	6.98	10.6	12.0	30	31.0	33.0	77.0	10.3	0.3	16
7	Before	6.51	8.6	11.5	32	22	24.5	63	8.0	0.3	31
	After 24 hrs.	5.21	12.4	13.0	31	29	30.0	53	7.7	0.2	25
Sacrif.	48 hrs.	6.12	8.4	9.5	35	29	38.0	64	7.3	0.9	18
8	Before	7.22	7.6	13.5	43	20	21.4	70	7.6	0.12	29
	After 24 hrs.	8.35	10.0	13.5	50	29	34.0	60	9.0	0.26	24
Sacrif.	48 hrs.	6.43	13.2	14.0	40	30	38.0	44	5.9	0.60	24
3	Before	6.31	7.6	14.0	40	20.0	23.9	84	11.4	0.48	18.0
	After 24 hrs.	6.21	11.6	14.5	42	29.0	36.0	90	10.4	0.30	16.0
	48 hrs.	5.62	9.1	10.5	36	31.5	37.5	74	7.4	2.20	13.0
Sacrif.	4th days	4.68	9.2	13.0	32	40.2	40.0	80	11.9	1.90	19.0
10	Before	6.22	6.8	12.5	34	21.4	22.0	57.0	11.9	--	30
	After 24 hrs.	7.12	14.2	15.0	46	28.0	29.5	80.0	8.8	0.26	17
	48 hrs.	6.86	11.2	14.5	45	29.0	35.5	47.0	8.3	0.60	19
Sacrif.	4th days	6.01	11.6	14.0	40	28.0	36.0	63.5	4.5	0.30	17
2	Before	7.19	9.2	14.0	50	22.0	25.1	80	7.13	0.24	17.0
	After 24hrs	6.10	13.4	11.5	35	29.0	36.0	90	10.4	0.30	16.0
	48 hrs.	5.32	8.6	9.5	32	32.0	39.0	57	7.6	2.40	16.7
	4th days	3.85	11.4	9.0	25	36.0	46.0	90	11.6	2.2	28.2
Died	6 days	-	-	-	-	-	-	-	-	-	-
5	Before	6.64	7.2	14.0	50	21.6	24.5	70	9.4	0.36	23.6
	After 24 hrs.	5.12	11.2	11.0	36	31.0	34.0	74	11.9	0.20	16.1
	48 hrs.	4.68	12.0	14.0	36	30.0	37.0	64	5.2	0.48	14.0
	4th days	4.32	11.8	13.0	34	29.5	39.0	67	4.5	0.42	14.0
Sacrif.	7 days	3.98	7.6	10.0	30	26.0	32.0	60	5.5	0.60	16.1
6	Before	3.61	7.4	8.0	20	22.0	25.1	67	5.6	-	28.0
	After 24 hrs.	3.32	14.4	8.0	22	29.0	30.0	77	7.4	-	21.0
	48 hrs.	3.01	13.6	8.0	15	29.5	36.0	44	4.9	0.24	22.0
	4th days	3.61	9.2	9.0	20	26.0	33.5	77	7.6	0.6	28.0
	7 days	2.00	15.4	4.0	11	27.0	39.5	37	7.4	1.3	26.0
Died	11 days	-	-	-	-	-	-	-	-	-	-
4	Before	8.98	8.4	15.0	55	25.0	24.0	84.0	7.4	0.30	30.8
	After 24 hrs.	5.62	12.8	11.5	35	31.5	34.5	63.0	7.4	0.20	17.3
	48 hrs.	5.16	9.8	12.0	35	31.0	41.0	47.0	6.5	0.42	19.0
	4th days	5.67	7.8	11.0	35	31.0	40.0	70.0	4.6	0.54	19.0
	7 days	5.06	7.8	10.0	30	27.0	33.5	67.0	6.1	0.10	37.0
Sacrif.	15 days	6.29	8.6	12.0	35	32.0	37.0	70.0	7.9	0.30	29.0

Table (III): Differential leucocytic count in blood of calves before and after infection with C.pseudotuberculosis

Duration	No. of Animals	Lymphocytes %	Monocyte %	Neutrophils %	Band-cells %	Eosinophlis %	Basophils %
Before infection	5	67.2(55-75)	5.6(3-8)	19.6(13-24)	1.75(7-2)	6(2-12)	0.2(0-1)
1st day of infection	5	63.2(58-71)	5.2(4-7)	23(17-27)	2.6 (1-4)	5.6(3-8)	0.2(0-1)
2nd day of infection	4	62.5(58-66)	6.3(3-10)	21.5(17-27)	4.5 (3-6)	5.1(3-7)	0.3(0-1)
4th day of infection	3	66(64-68)	4.3(3-5)	20.7(19-22)	4.7 (3-7)	3.7(2-5)	0.2(0.1)
7th day of infection	2	64.5(59-70)	7.3(5-10)	76(14-18)	5.5 (3-8)	6(468)	0.5(0.1)
15th day of infection	1	62	10	18	7	3	0

Table (IV)

Differential leucocytic count in buffaloe calves before and after infection with C.ovis

Time	No. of animals	Lymphocyte %	Monocyte %	Neutrophil %	Band cell %	Esinophil %	Basophil %
Before	10	65.7(61-73)	7 (5-9)	18.7(13-26)	2 (1-3)	6.2(2-12)	0.2(0-1)
After One day	10	61.3(56-67)	7.4(5-10)	22.8(19-27)	4 (2-5)	5 (3-8)	0.2(0-1)
After 2 days	8	58.3(54-62)	6.0(3-9)	24.5(20-29)	3.5 (2-5)	6.3(2-10)	0.3(0-1)
After 4 days	6	64 (60-69)	6 (4-8)	22.5(17-26)	4.3 (2-6)	4 (2-6)	0.2(0-1)
After 7 days	3	66 (63-68)	8.3(6-10)	18.7(17-20)	5 (4-6)	5 (3-7)	0.3(0-1)
After 15 days	One	58	12	20	5	5	0 (0-0)

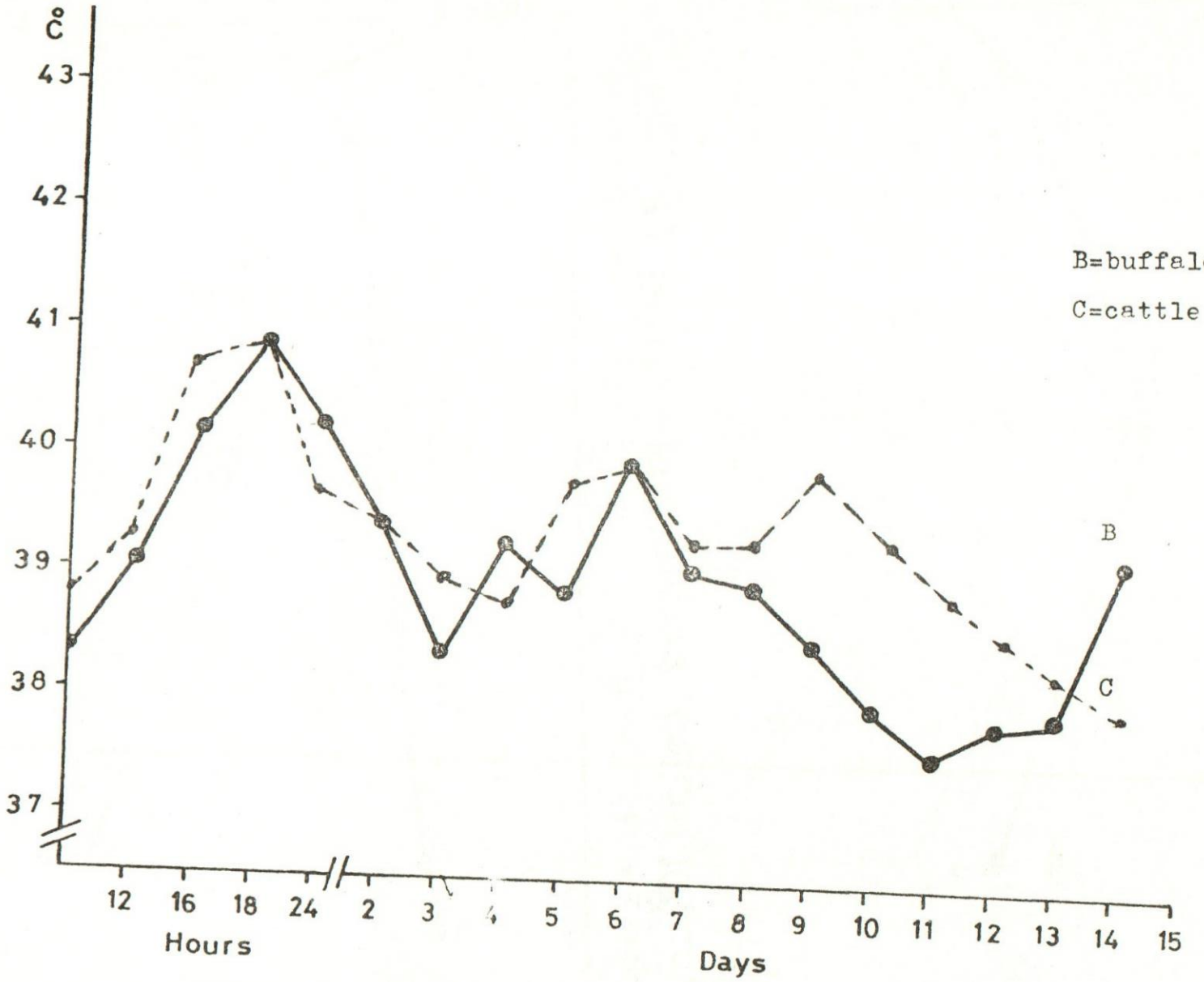

Fig.(1) Temperature curve.

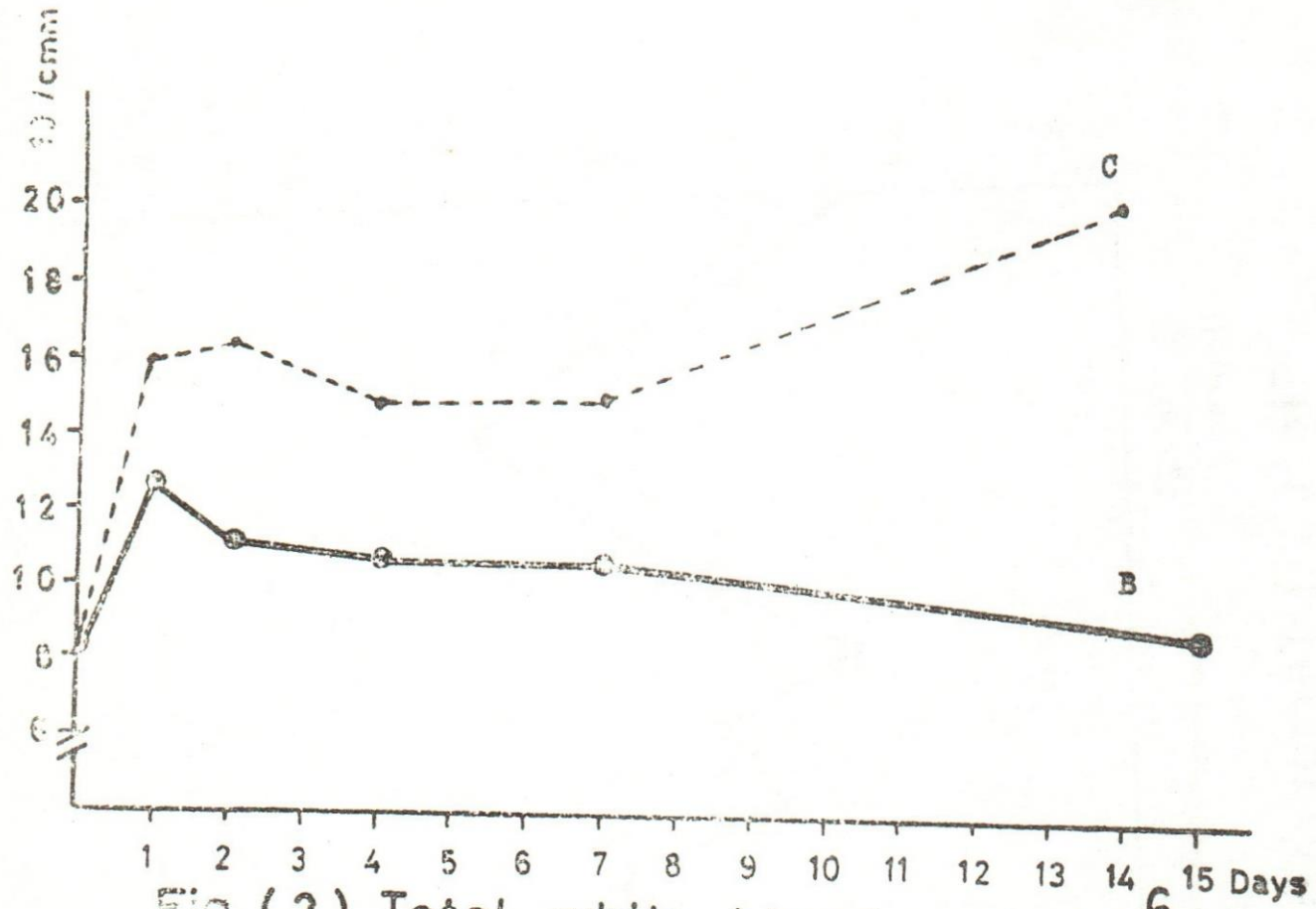

Fig. (2) Total white blood cells (10^6/cmm

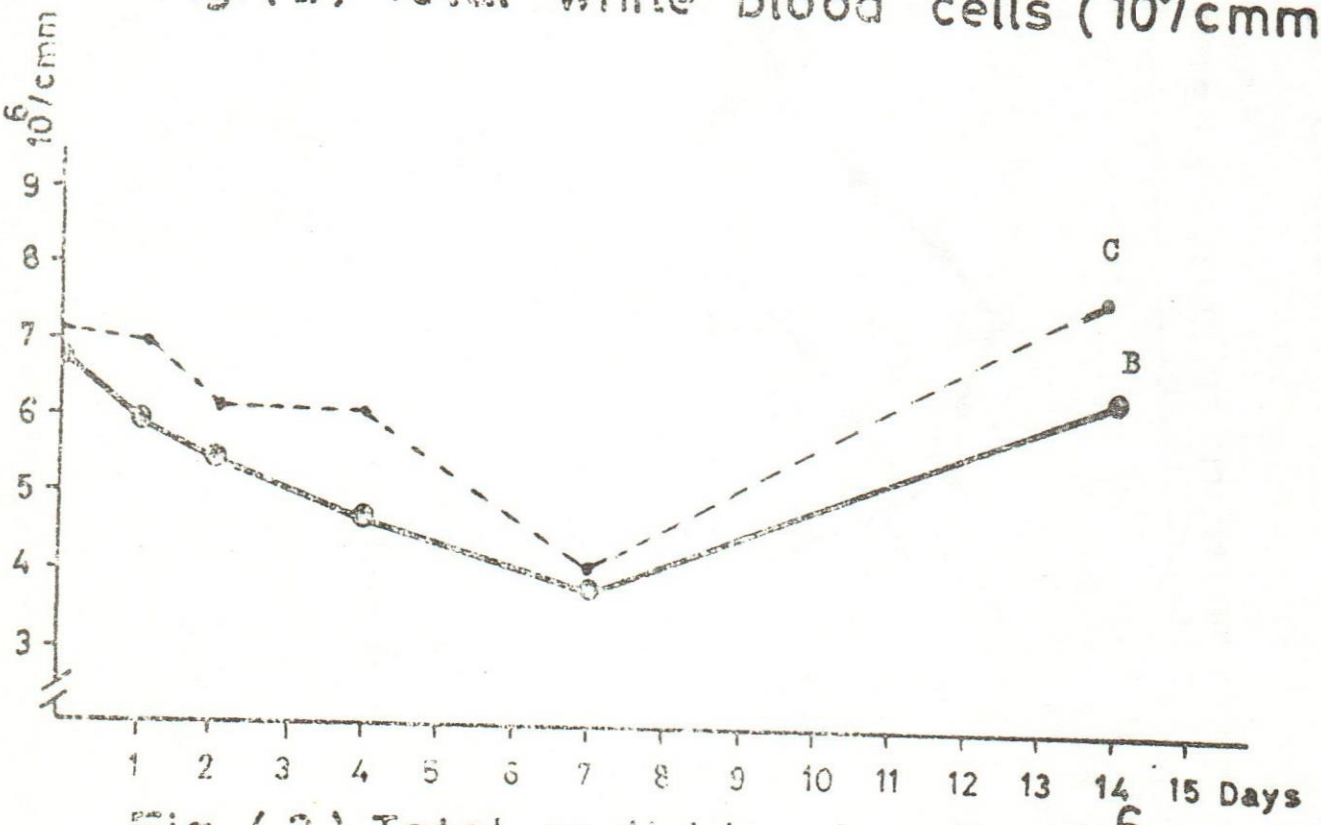

Fig. (3) Total red blood cells (10^6/cmm).

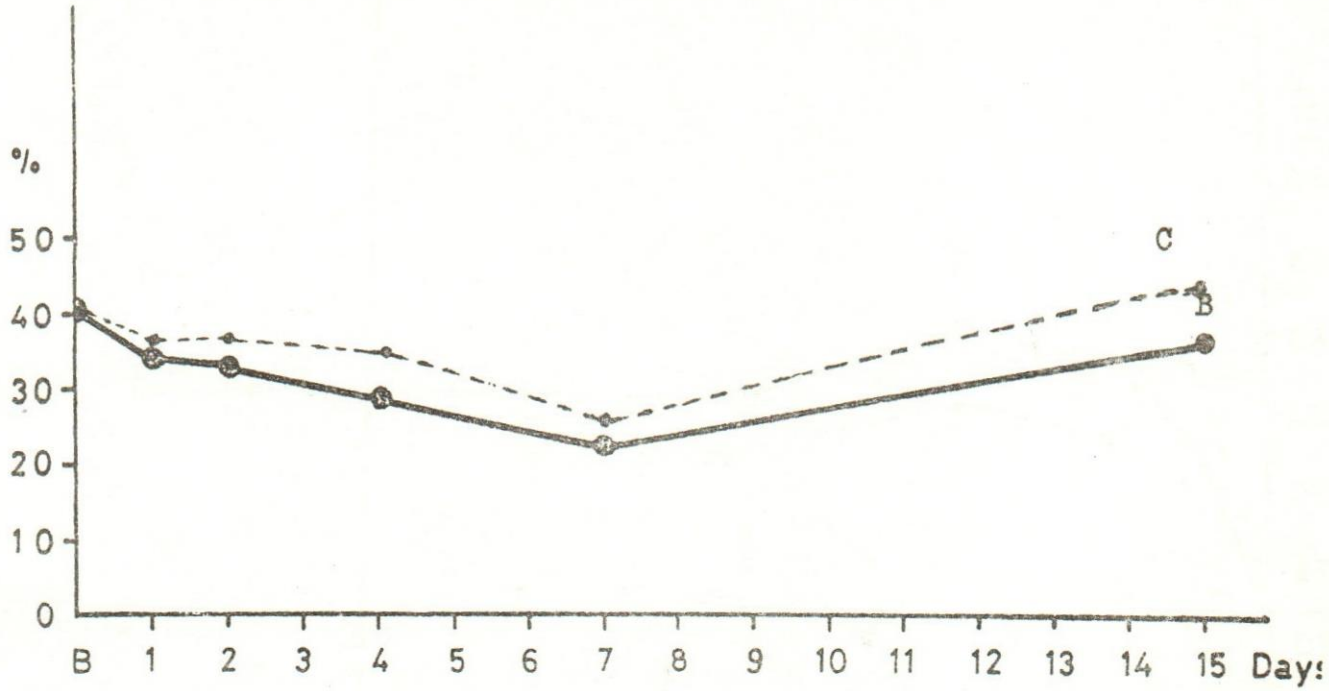

Fig.(4) Packed cell volume .

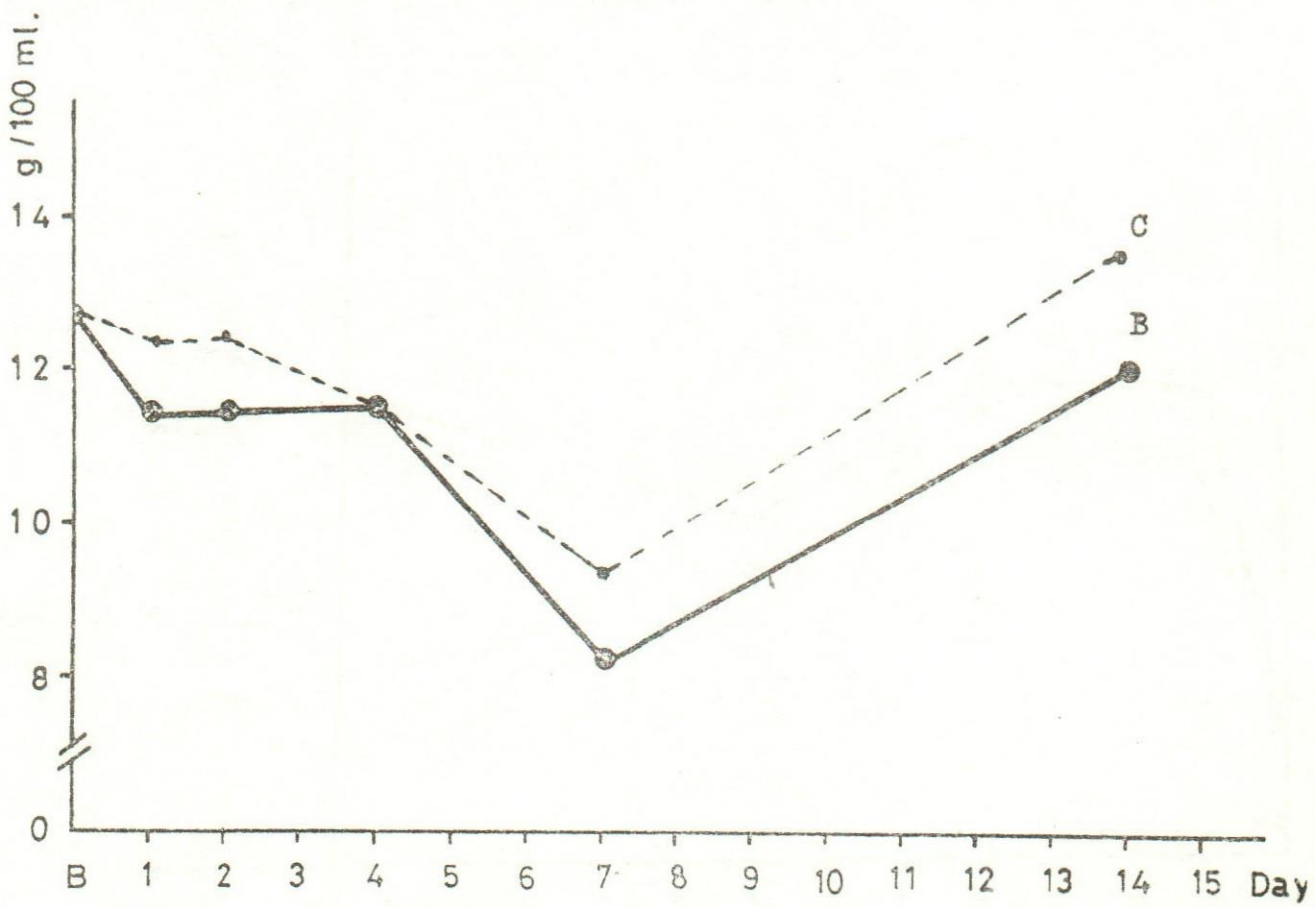

Fig. (5) Hemoglobin.

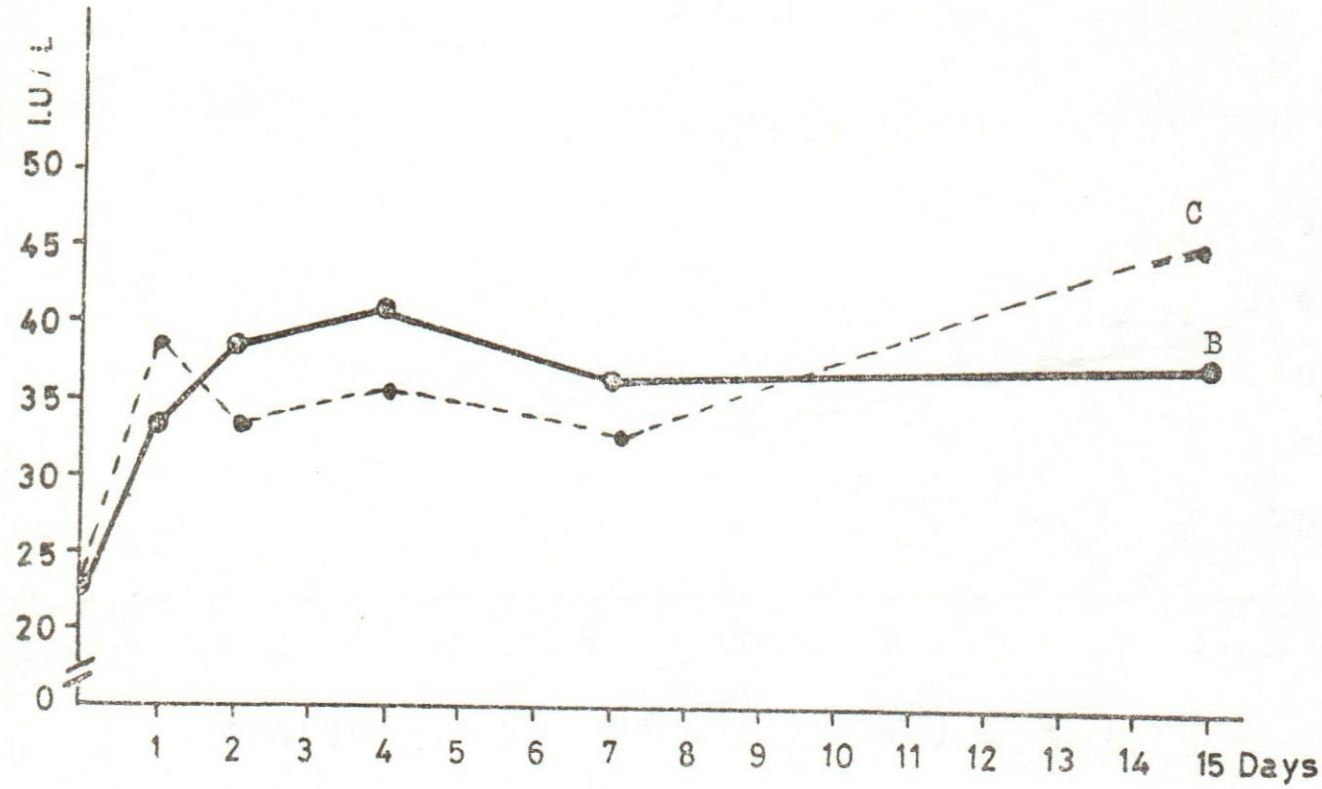

Fig. (6) Glutamic oxaloacetic transaminase

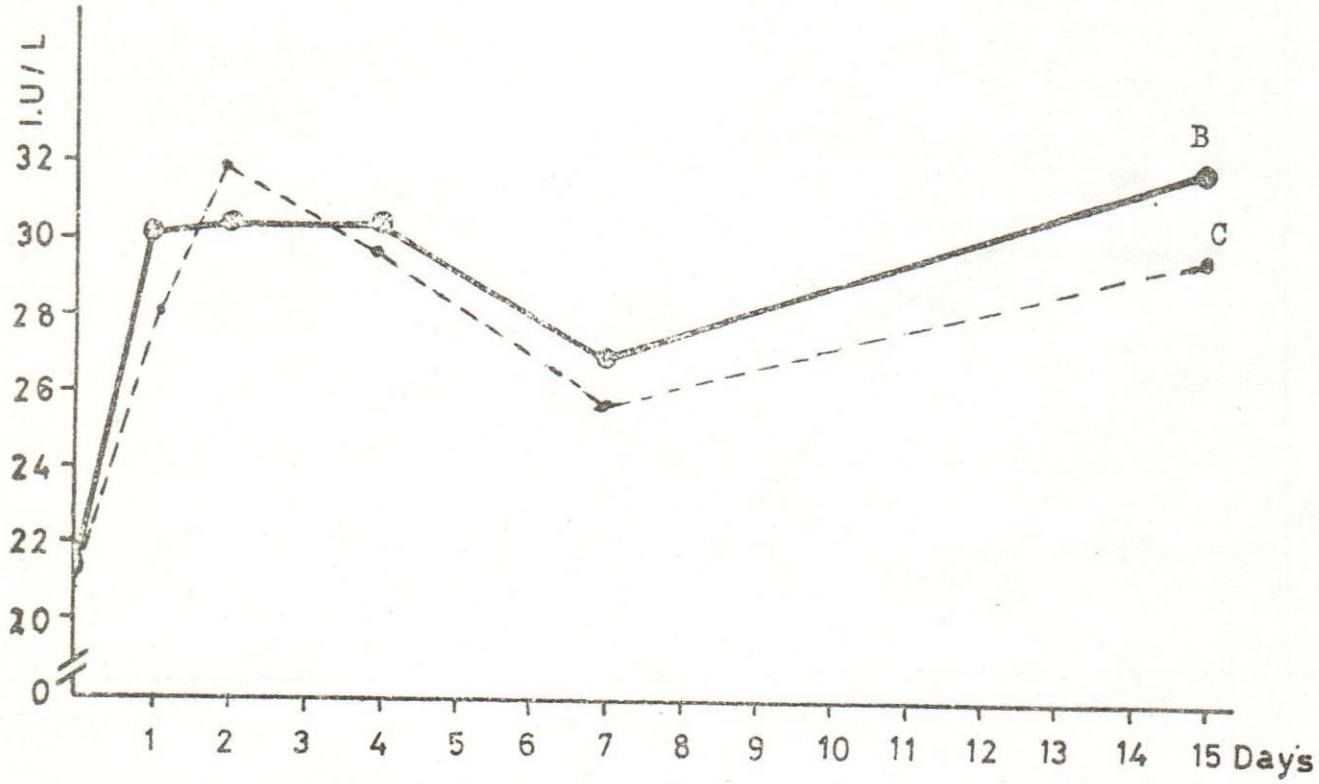

Fig. (7) Glutamic pyruvic transaminase.

Fig. 8. Blood urea nitrogen.