قسم : طب الحيوان العام.
كلية : الطب البيطرى - جامعة أسيوط.
رئيس القسم : أ.د./ابراهيم سيد احمد .

نمط التحليل الكهربي لبروتين مصل الدم تحت تأثير الاصابة الفيروسية لمرض التهاب القصبة الهوائية والاسهال الفيروسي في عجول التسمين

عماد نافع ، نورالدين حسان ،على السباعي

اجرى هذا البحث على عدد ٣١ رأس من السلالات المحلية من عجول التسمين تتراوح أعمارها مابين ٦-١٨ شهرا ، ثماني عجول من هذه الحيوانات كانت العلامات المرضية المميزة لمرض القصبة الهوائية والاسهال الفيروسي واضحة عليها ، ثماني عجول أخرى كانت فقط درجة حرارتها مرتفعة وبدون وجود علامات مرضية أخرى . ولقد اختيرت كذلك ثماني عجول من التي في مرحلة نقاهة واجتازت الحالة المرضية الحادة .

أخذ في هذا البحث ٧ عجول سليمة وخالية من أية علامات مرضية .

ولقد أشارت نتائج التحليل الكهربي لبروتين الدم أن شق الالبيومين في جميع المجموعات التي احتواها البحث لم يتأثر معنويا ، بينما لوحظ ارتفاع مميز في نسبتين الالفا (١) ، (٢) بالنسبة لشق الجلوبيولين في المجموعات المريضة ، كان هناك نقص واضح في كمية البيتا (٢) لمشتق الجلوبيولين في المجموعة الرابعة ،بينما لم تسجل القراءات أى معدلات في المجموعة الثانية والثالثة ، أما بالنسبة لمشتق الجاما جلوبيولين فقد سجلت النتائج نقص واضح ملحوظ في المجموعة الثانية والرابعة ، بينما اختفى كلية في المجموعة الثالثة .

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SERUM PROTEIN ELECTROPHORESIS PROFILES UNDER SPONTANEOUS CASES OF INFECTIOUS BOVINE RHINO-TRACHEITIS (IBR) AND MUCOSAL DISEASE VIRUS DIARRHOEA (MD - VD) INFECTION IN FATTENING CALVES

(With One Table and 6 Figures)

By
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SUMMARY

This work was conducted on a ttoal 31 calves of the native breed, their age were between 6 and 18 months. Seven animals showed the typical IBR. MD - VD virus infection symptoms. Eight calves showed severe febrile conditions without exhibitin the apparent clinical signs and another eight calves seem to be in the convalescent stage and seem to show symptoms of recovery. Eight calves were kept as control throughout the whole time of the study. Serum protein electrophoretic values are presented for the clinical apparently healthy calves and the other groups, using a standardized method for protein analysis and fractionation.

No significant cahnges is either relative or absolute amounts of serum proteins and albumin were detected in the serum of the affected calves when compared with the presumably, healthy calves. Highly significant rise in the 1 and 2 globulin fractions was observed in all affected groups indicating severe inflammatory processes accompanied by necrosis. Severe depression of B-2 glublin fraction was observed in the convalescent group, while total absence of the same fraction in both diseased and feverish group was evident.

INTRODUCTION

Stations of fattening calves have a problem that stems from a respiratory disease and it is irrefutable that these diseases are amongest the most important ffecting fattening calves throughout the world (PIRIE, 1978). Some of problems are compartively trivail, others, may constitute a major cause of economic catastrophas in cattle industry (ANDREWES et al. 1981).

The causes of respiratory affections in fattening calves were identified by many authors throughout the world. Infectious Bovine Rhinotracheitis (IBR), Bovine Adeno Virus, Bovine Virus Diarrhoea (BVD), Syncitial Respiratory Virus and Para influenza virus type- 3 were identified as the most important viruses causing respiratory diseases in cattle (AMSTUTZ, 1982).

The importance of IBR in Egypt has increased dramatically in the last year when the virus ws isolated from cases associated with Pasteurella multocida in buffalo calves (EL-SEBAIE et al. 1984).

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Most of the studies that have been conducted in veterinary medicine have involved the plasma proteins in foot and mouth disease, vesicular stomatitis, hog cholera, New castle disese and Infectious brochitis (CORNELIUS and KANEKO, 1968).

In our local circumstances, a heavy demand have been increased in the number of requests for serum protein electrophoresis as a diagnostic aid to reach a correct diagnosis of viral infections in fattending stations.

Meagre informations are available concerning the electropheratic pattern of serum proteins in upper respiratory affections in calves. So it is aimed in this investigation to throw the light on the effect of IBR- MD/VD virus infection on the immune system of the calves affected. This will help much in the diagnosis of such diseses which mostly prevalent in feedlot.

MATERIALS and METHODS

ANIMALS:

Thirty-one calves for the present study after notification were selected and classified according to the clinical situation of the animals. Their age were from 6 - 18 months of both sex and thier weight ranged from 150-300 kg. The first group included eight calves presumably clinically healthy and kept as control throughout the study. The second group comprised seven animals, they did show the typicl symptoms of the IBR, MD/VD virus infection. The third roup, being eight calves showed a high rise of body temperature since four days before selection as well as the classical sins of the disease. The fourth group included eight animals selected out of the herd that known to be treated and given antibiotic and antipyretic regimen. The diseased calves were selected from a herd of 200 head suffered an oubreak of IBR, MD/VD virus infection, while the other presumably healthy group belonged to a separate healthy farm, both Stations were at El-Nekheila district in the near south of Assiut Province.

BLOOD SAMPLES:

Samples of blood were taken from the 31 animals using jugular veinpuncture, were allowed to clot at room temperature before the serum was separated by centrifugation. The serum samples were dispensed into plastic containers labelled and stored at 4°C for 2 - 4 days, where electrophoresis was performed within this period.

TOTAL PROTEINS:

Were estimated using test kits supplied by Boehringer Mannheim (W. Germany). The final absorbances were read in 1 cms cuvettes against a reagent blank at 546 nm in a Beckman-25 uv/visible range digital spectrophotometer.

ELECTROPHORESIS:

Electrophoresis of serum proteins was carried out according to the procedure described by the manufacturer (Corning ACL film/ cassette electrophoresis system- application manual). Of each serum sample 0.8 ul was laded into preformed, numbered sample wells on the agarose film. Each film cold accomodate up to eight serum samples. The films were electrophoresed for 35 minutes at a constant 90 volts with fresh, refigerated barbital/EDTA buffer solution (PH 8.6, ionic strength 0.05 M). After electrophoresis the films were simultaneously fixed and stained in an acetic acid-amido-black stain solution for 15 minutes then dried at 37°C.

After destaining in acetic acid and drying completely, the films were scanned densitometerically at 525 nm in a. Gelman DCD-16 scanning densitometer.

VIROLOICAL ISOLATIONS:

Pahological materials were collected from dead animals show typical clinical manfestation for laboratory diagnosis at the Dept. of Virology, Institute of Animal Health, Dokki, Cairo. In the same time laboratory samples were sent to Dept. of Virology, Giessen Univesity, West Germany, for confirmation. These pathological materials were in form of serum, ocular discharge, tracheal secretions, nasal swabs, lymph node, small intestine, and part of a trachea.

RESULTS

The most common clinical findings in these incidents were nasal discharge with congestion of the nasal mucosa, ocular discharge with conjunctivitis, coughing, dullness, reduced appetite to complete inappettance and long standing fever, in some cases there were soft fasces to profuse bloody diarrhoea. With the progress of the illness animals became dehydrated and loss their body weight.

Results of the laboratory diagnosis of the causative agents revealed the responsibility of IBR-M/VD for the production of such infection, as the laboratory of Dokki reported the isolation of causative agent IBR from the ocular discharge and nasal swabs. Serological investigation on serum samples collected denoted the presence of a significant titre of antibody against IBR. BVD which confirmed the existence of infection with such group of viruses. Additional and confirmatory viral isolations were carried out on the similar samples in Depts. of diagnostic virology in, Giessen Univ. W. Germany.

DISCUSSION

Concerning the total serum proteins and albuming fraction, the data obtained showed no significant variations in either both total protein or albumin in the groups examined. These results are in full agreement with those of DIMOPOULLAS (1961) who stated that viral infections do not produce significant changes in either relative or absolute amounts of plasma protein, even through the antibody titres may be elevated.

A marked increase of a-1 ($P/\_\_$ 0.01) gloublin fraction was observed in the diseased and feverish groups as well as in the convalescent one. Similar results were recorded by SCHALM (1975) who explained such elevation probably to adrenal stimulation and protein catabolism following the diseased condition.

Highly significant increase in a-1 and a-2 globulin fractions in the convalescant group (30.50 $\pm$ 5.05, 16.55 $\pm$ 8.35), was evident when compared with the other diseased and feverish groups (Table 1 and Figs. 1, 2, 3, 4, 5 & 6). A resonable explanation for this increase could be referred mostly to a massive tissue destruction that accompanied necrosis. These results were in agreement with those of AFFONSO et al., (1960).

Beta-1 and beta-2 and Gamma-globulin, fractions showed a highly decrese in their concentration in the convalescent group, to complete absence of these fractions in the diseased (II and feverish II) groups (Table 1, Figs. 1,.2, 3, 4, 5 & 6). From the clinical and pathological point of view, it is well known that the infection wih IBR and MD/VD is mostly accompanied with massive tissue destruction in the upper respiratory and the alimentary tract as well. In the mean time the destructive action affect the immune system of the disesed animal

leading to immune suppression. Such destructive processes could explain these findings which were previously declarified by LOMBA (1976) and OBI et al., (1981). This immuno suppressive action on the beta and gamma globulin fraction in our results are in consistent with those reported by SCHALM (1975) who stated that the first antibodies to appear in the plasma after most antigenic exposure are globulins of beta mobility. Previous interpretation for similar findings stated by that author indicated that the causative organism may induce damage to the b-2 and 9-globulin producing cells or it may cause damage to small blood vessels and allow serum proteins to escape into tissues.

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SERUM ELECTROPHORESIS UNDER IBR AND MD INFECTION

TABLE (1): Serum jprotein pattern (Mean ± SDM) of apparently healthy, diseased, feverish and convalescent calves, naturally infected with IBR, MD/VD virus.

	N	Total proteins g/L	Albumin %	Globulin a-1 %	a-2 %	b-1 %	b-2 %	g
Group I	8	97.6	46.47	09.85	09.26	17.52	09.79	09.56
(Presumably healthy)		±2.30	±3.10	±0.82	±1.69	±3.28	±3.47	±3.29
Group II	7	98.1	50.44	*** 24.23	*** 14.93	*** 06.49	*** ---	*** 03.97
(Diseased)		±0.20	±2.92	±3.78	±2.81	±3.66	---	±1.55
Group III	8	92.80	51.36	*** 28.03	*** 14.59	*** 05.41	*** ---	*** ----
(Feverish)		±1.30	±24.10	±3.45	±2.13	±1.92	---	---
Group IV	8	94.7	42.40	*** 30.50	*** 16.55	*** 07.80	*** 00.93	*** 00.87
(Convalescent)		±1.3	±5.67	± 5.05	± 8.35	±5.66	±0.94	±1.52

*** = high significance or $P < 0.01$.

N = number of animals.

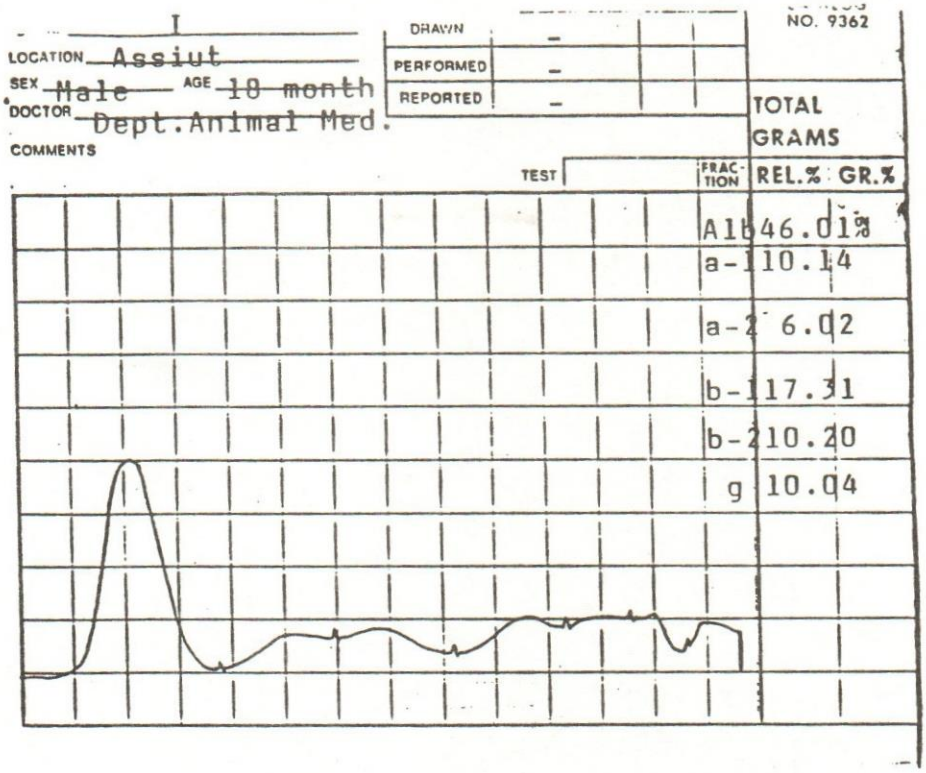

Fig.1. Electrophoretic pattern of serum proteins in presumably healthy calves.

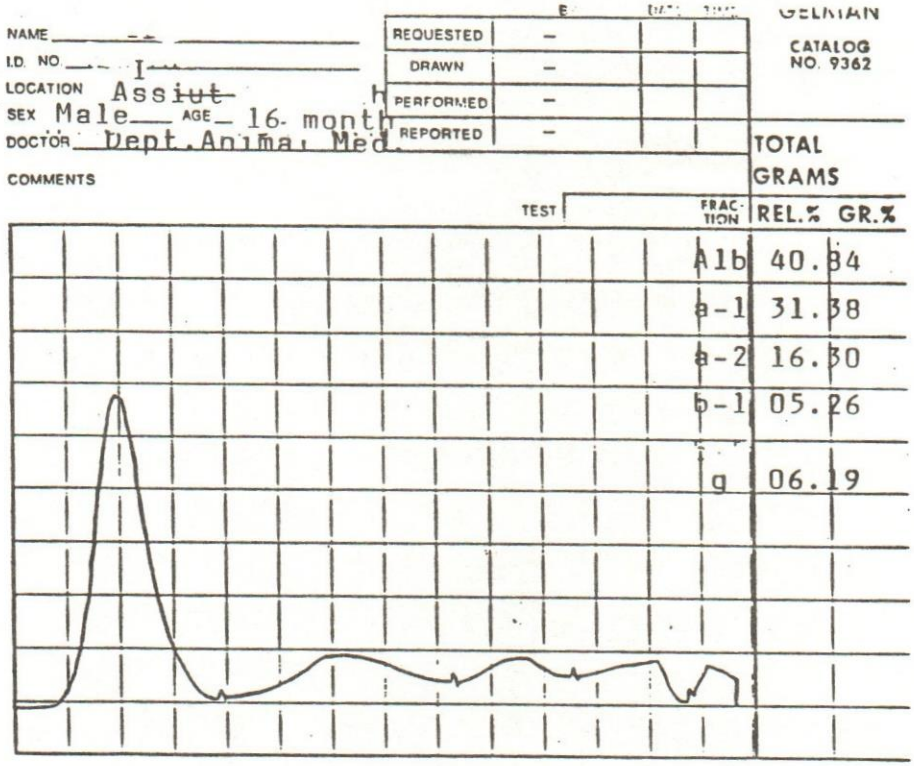

Fig.2. Electrophoretic pattern of serum proteins in calves suffering IBR MD-VD virus infection (Diseased cases).

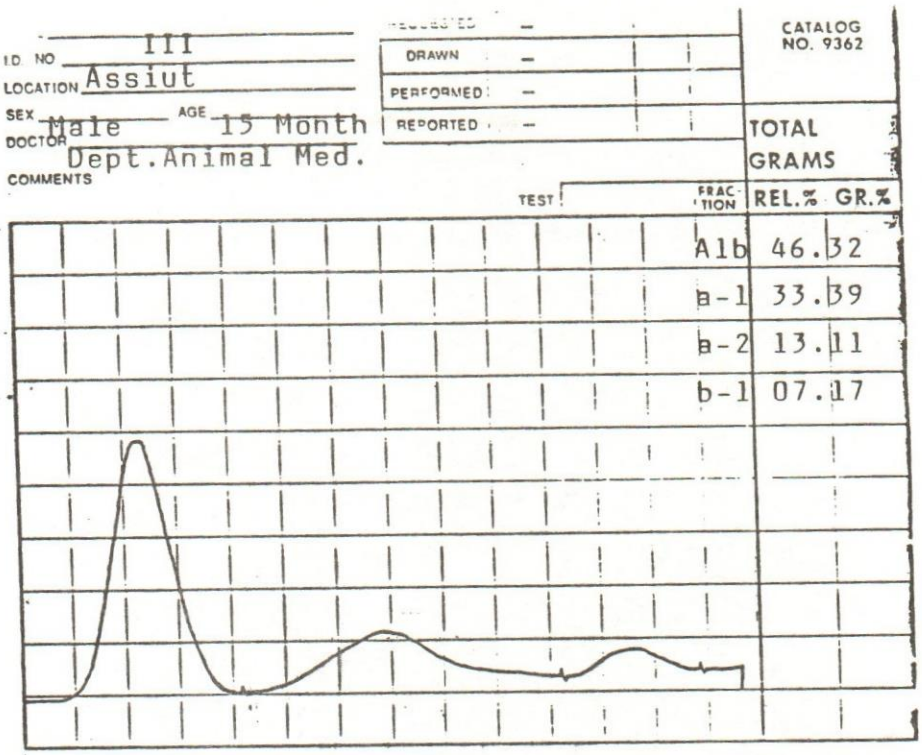

Fig.3: Electrophoretic pattern of serum proteins in calves showing fever(early stage infection).

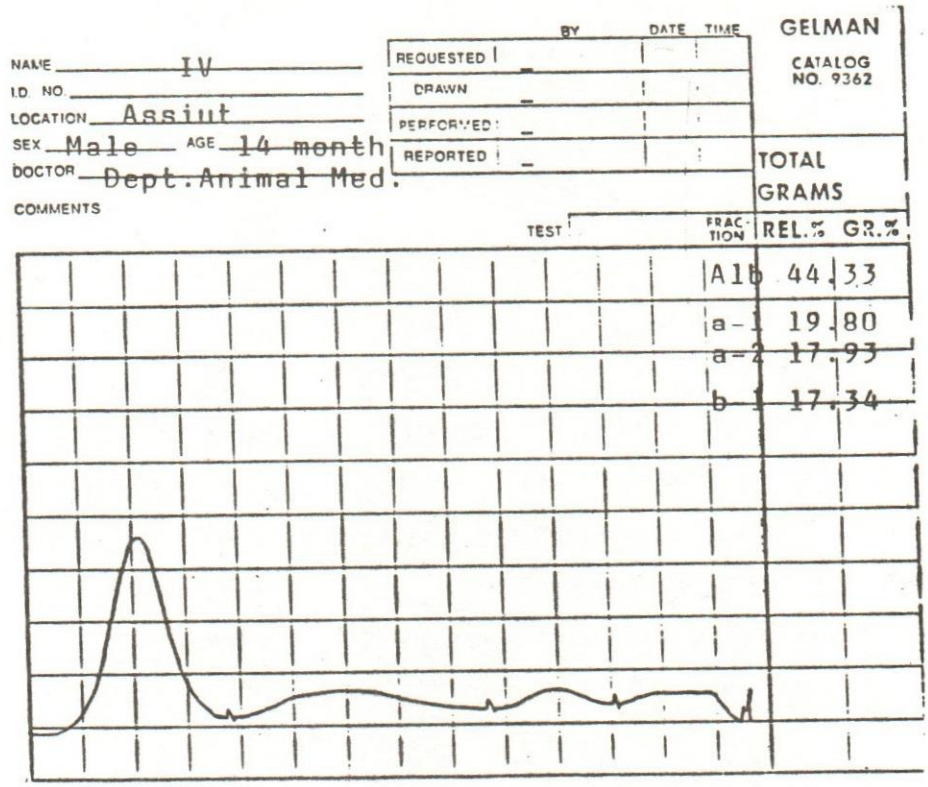

Fig.4. Electrophoretic tracing of serum proteins in convalescent calves.

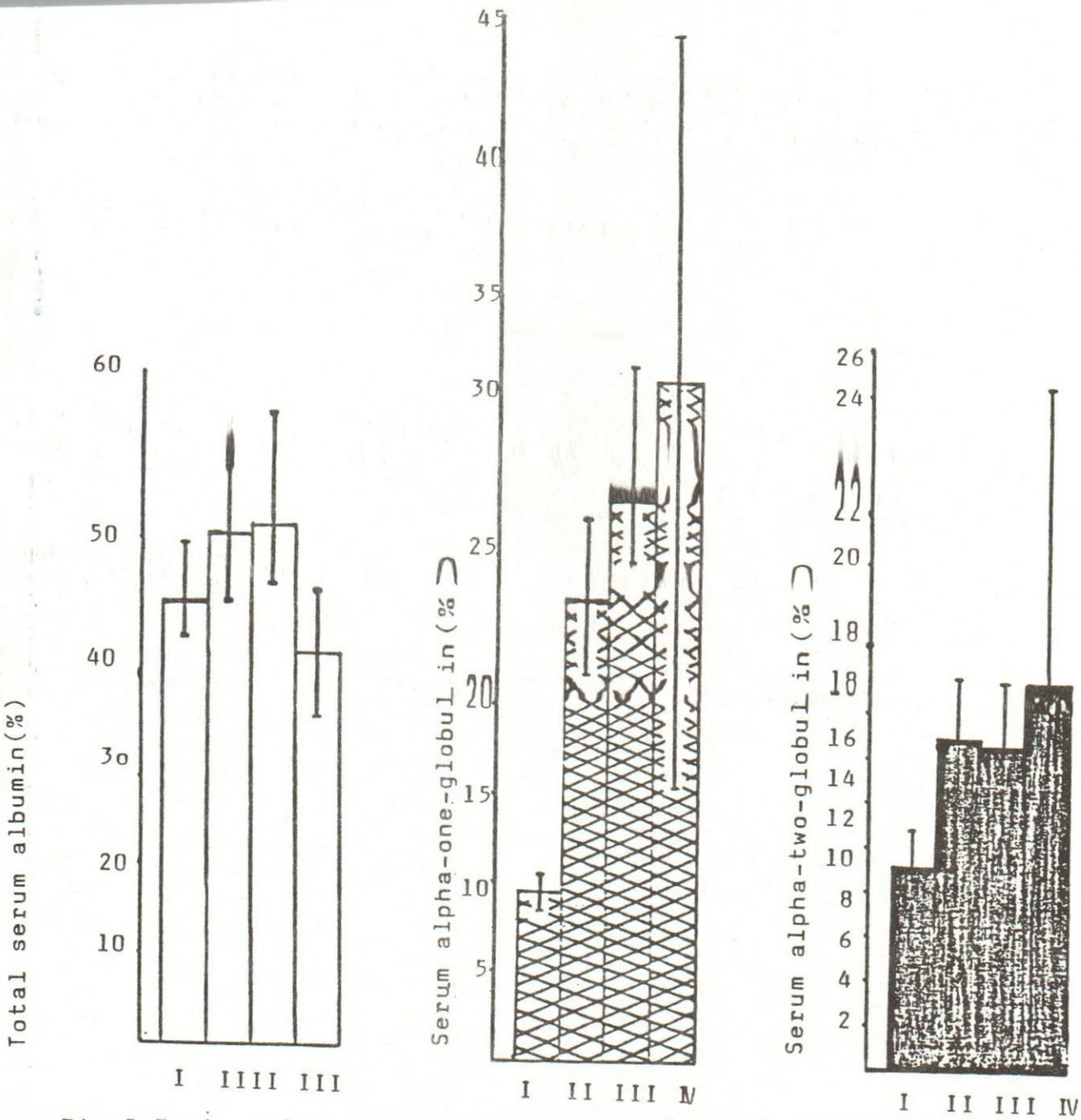

Fig.5.Empty columns mean percentages of total serum albumin(mean± SOM),cross-hatched columns mean percentages of serum a-1-globulin(mean±SOM),& a-2-globulin(mean± SDM), I,II,III,IV mean the presumably healthy , diseased,feverish 1 convalescent group respectively.

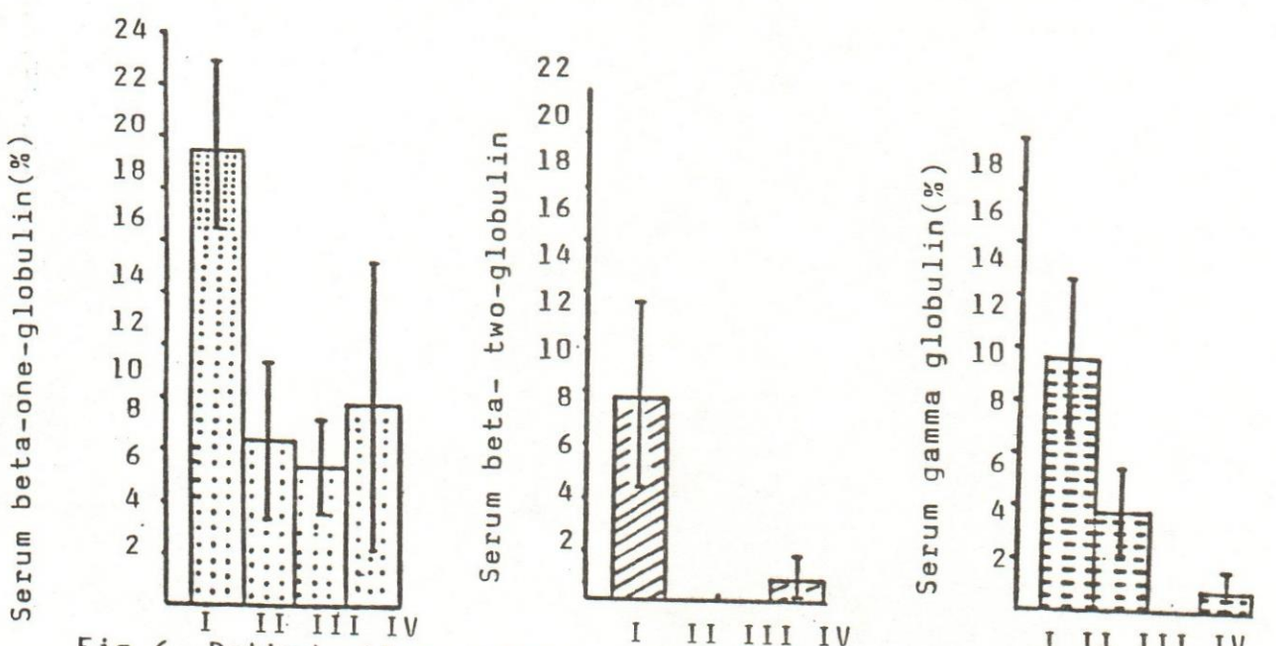

Fig.6. Dotted columns mean percentages of b-1-globulin(mean±SDM), hatched columns mean percentages of b-2-globulin(mean±SDM), interrupted lines mean percentages of g-globulin(mean+SDM). I,II,III,IV as fig. 1.