EFFECTS OF VARYING LEVELS OF CRUDE PROTEIN ON SOME BLOOD PARAMETERS AND IMMUNE RESPONSE IN SHEEP

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This is an original work carried out by myself and no part of it has been presented elsewhere for a degree or diploma.

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DEDICATION

Dedicated with love to

my most able partner

RUTH

and our girls

ANNA and JOANNA

ABSTRACT

Effects of different levels of crude protein on weight changes, some blood parameters and immune response were investigated in eighteen Yankasa ewes in three groups of six. Their diet contained 15% (high), 7.5% (medium) and 4% (low) crude protein respectively.

Daily weight gains were highest for the animals on the high protein diet (103.7 grams) as compared to those on medium (81.3 grams) and low protein diets (53.7 grams).

The packed cell volume varied significantly ($P<0.05$) between the groups. The means and standard deviation of PCV values in ewes fed high, medium and low protein diets were 34.0 ± 1.0, 29.5 ± 2.3 and 24.0 ± 2.5 grams percent respectively. Though the hemoglobin values did not show a statistically significant change between the groups ($P>0.05$), the mean values at several sampling points were highest for the animals in the high protein group, indicating a nutritional effect.

The total white blood cell count was not significantly affected by the diet ($P<0.05$). Also no significant differences were observed on the

absolute lymphocyte, eosinophil and monocyte counts. But the neutrophil count was significantly affected with the animals on the low protein diet having a higher mean value than those on the medium and high protein diets.

Serum total protein and the gamma globulin fraction were significantly higher ($P<0.05$) in the animals on high protein diet than in those on the medium and low. Serum albumin levels, $Alfa_1$, $Alfa_2$ and Beta globulin levels were not significantly affected by diet.

Animals in the high and medium protein groups had significantly higher ($P<0.05$) antibody titer two weeks post inoculation with the salmonella vaccine. At three weeks post inoculation, the three experimental groups had significantly different antibody titer. At eight weeks post inoculation, only the high protein group had significantly higher antibody titer as compared to the medium and low.

It is concluded from this study that dietary protein has significant influences on weight gains, packed cell volume, peripheral absolute neutrophil counts, total serum protein concentrations and the gamma globulin fraction in Yankasa ewes and that production of specific antibodies may be compromised in circumstances where the dietary protein is low.

TABLE OF CONTENT

TITLE PAGE	Page
DECLARATION	i
ACKNOWLEDGEMENTS	ii
DEDICATION	iv
ABSTRACT	v
TABLES OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	xi
INTRODUCTION	1
Chapter I	
LITERATURE REVIEW	5
The role of protein in the body ..	6
Nutrition and the blood profile ..	8
The structure and function of the immune system	10
Malnutrition and disease	14
Effects of protein malnutrition on the immune system	15
Chapter II	
DIETARY PROTEIN: EFFECT ON SOME BLOOD PARAMETERS AND WEIGHT GAINS IN YANKASA EWES	20
Introduction	21
Materials and Methods	22

Results 24

Discussion 25

Chapter III

DIETARY PROTEIN: EFFECTS ON SERUM PROTEIN FRACTIONS AND IMMUNE RESPONSE IN YANKASA EWES 28

Introduction 29

Materials and Methods 30

Results 31

Discussion 33

Chapter IV

GENERAL SUMMARY AND CONCLUSIONS 36

REFERENCES 40

FIGURES 49

TABLES 55

APPENDIX 74

LIST OF TABLES

Table 1:	Percentage composition of Experimental rations	55
Table 2:	Proximate Analysis of Experimental Rations	56
Table 3:	Effects of different levels of dietary protein on some blood parameters and weight changes in sheep	57
Table 4:	Means±SD for packed cell volume of ewes fed different levels of crude protein (%) ..	58
Table 5:	Means ± SD for hemoglobin of ewes fed different levels of crude protein (gm/dl)	59
Table 6:	Means ± SD for eosinophils of ewes fed different levels of crude protein	60
Table 7:	Means ± SD for monocytes of ewes fed different levels of crude protein	61
Table 8:	Means ± SD for lymphocytes of ewes fed different levels of crude protein	62
Table 9:	Means ± SD for neutrophils of ewes fed diffeent levels of crude protein	63
Table 10:	Means ± SD for Total leucocytes of ewes fed different levels of crude protein	64
Table 11:	Bacterial tube agglutination titres of ewes fed different levels of crude protein innoculated with salmonella vaccine	65

Table 12: Effects of different levels of crude protein on total serum protein and serum protein fractions 66

Table 13: Means ± SD for serum total protein of ewes fed different levels of crude protein (gm/dl) 67

Table 14: Means ± SD for serum total protein of ewes fed different levels of crude protein (gm/dl) 68

Table 15: Means ± SD for $Alfa_1$ globulin of ewes fed different levels of crude protein (gm/dl) .. 69

Table 16: Means ± SD for $Alfa_2$ globulin of ewes fed different levels of crude protein (gm/dl) .. 70

Table 17: Means ± SD for Beta globulin of ewes fed different levels of crude protein (gm/dl) .. 71

Table 18: Means ± SD for gamma globulins of ewes fed different levels of crude protein (gm/dl) .. 72

Table 19: Means ± SD of total globulins of ewes fed different levels of dietary protein (gm/dl) 73

LIST OF FIGURES

Figure 1: Effects of different crude protein level on packed cell volume .. 49

Figure 2: Effects of different crude protein levels on hemoglobin 49

Figure 3: Effects of different crude protein levels on total white blood cell 49

Figure 4: Effects of different crude protein levels on serum total protein 50

Figure 5: Effects of different crude protein level on serum albumin 50

Figure 6: Effects of different crude protein level on total serum globulins 50

Figure 7: Effects of different crude protein levels on $Alfa_1$ globulin 51

Figure 8: Effects of different crude protein levels on $Alfa_2$ globulin 52

Figure 9: Effects of different crude protein levels on Beta globulins 53

Figure 10: Effects of different crude protein levels on gamma globulins 54

LIST OF ABREVIATIONS

%	-	PERCENT
PCV	-	PACKED CELL VOLUME
Hb	-	HEMOGLOBIN
CP	-	CRUDE PROTEIN
DM	-	DRY MATTER
N_2	-	NITROGEN
EE	-	ETHER EXTRACT
CF	-	CRUDE FIBER
ANOVA	-	ANALYSIS OF VARIANCE
TP	-	TOTAL PROTEIN
SD	-	STANDARD DEVIATION
g/dl	-	GRAMS PER DECILITER
Umole	-	MICRO MOLE

INTRODUCTION

There is no accurate statistics in Nigeria with regards to the total livestock population, but sheep population has been put at eight million (David West, 1975).

Sheep play an important role in the socio-economic and cultural life of the people of Nigeria. Besides being one of the major sources of meat, they also make a significant contribution to national economy. Sheep are valuable assets to the local populace to whom the small stock is an intergral part of the family unit and an emergency source of fund (Adu and Ngere, 1979).

In Nigeria, 81.9% of households own one type of livestock or the other and 66.8% of these household owning stock varies from 2.8 in areas of light density to between 6 and 10 in high density areas (Fos, 1970).

Studies conducted (Adu and Ngere, 1979) showed that certain parameters such as; reproductive performance, birth weight, growth rate, meat production and milk yield are low when compared to sheep reared in temperate regions.

Causes of low productive life of sheep indigenous to Nigeria may be due to a combination of one or more factors such as; environment, genetic, disease, and nutritional influences. Since the livestock industry in Nigeria is struggling to meet increasing demand for meat, it will therefore be necessary to examine all factors that affect production, not only in large ruminant but also in the smaller ones.

In the few government sheep farms in Nigeria, modern system of husbandry is practiced but over 60% of the total sheep population in Nigeria are reared by nomadic herdsmen in the Sudan and Sahel regions of the country where availability of pasture varies with the season. It has been reported that pasture during the hot dry season may have less than 4% crude protein level (Miller and Blair, 1963). The nomadic herdsmen who rear most of these animals have kept to traditional methods and lack any incentive to change to modern management practices. Hence sheep production is still in very rudimentary stages of development.

Reports have been made that disease are most times compounded by malnutrition and have hindered the economic production of sheep in Nigeria (Akerejola et al., 1979).

This inadequacy in diets either predisposes to or exacerbates infectious disease as has been verified in many experimental models (Watson, 1937; Schaedler and Dubos, 1959; Boyd and Edwards, 1963; Scrinshaw et al., 1968). Several host defensive mechanisms are affected in malnourishment. In particular, inflammatory responses (Taylor et al., 1977), phagocytic capacity (Guggenheim and Buechler, 1948; La via et al., 1956) and antibody production (Renney et al., 1968; Aschkenasy, 1973; McFarlane and Hamid, 1973) have all been found to be either reduced or absent in protein deficient host.

Immunecompetence has been said to be a sensitive indicator of the adequacy of nutritional regimens (Sheffy and Williams, 1982). Though protein malnutrition in Nigerian animals has been mentioned by different workers, no work has been done on the immunecompetence of the Nigerian sheep in relation to the rations fed under intensive system of management or the traditional system. This work was therefore designed to examine three planes of dietary protein on the immune response of weaned Yankasa ewes (levels close to those practiced under the different husbandry systems) and to examine the effects of these rations on some haematological parameters and weight changes.

Chapter I

LITERATURE REVIEW

The Role Protein in the Body

Fundamentally, it does not seem logical to attempt to rank the nutrients animals require in terms of importance. If they are requirements, they must all be present and the result of deficiencies are undesirable (Crampton and Haris, 1968). From the standpoint of practical feeding however, and especially in the sense of daily requirements, we can rank nutrients in the order of their importance.

For full growth, the organism requires all essential nutrients, but protein is considered limiting (Miller and Mitchell, 1969).

Deficiency of dietary protein during growth and development interferes with the maturation of all organs and tissues. Of the six major categories of nutrients (carbohydrate, fat, protein, vitamins, minerals and water) protein is the most important especially early in life (Cooper et al., 1974). During this time, the organism accumulates in a programmed and regulated manner, body proteins of many types and functions which are essential to its metabolism. Without a balanced input of amino acids, the suckling mammal's growth and development are compromised, probably permanently (Good and Jose, 1975). It has also been reported that protein

deficiency results in poor mental health in humans (Scrimshaw et al., 1968).

Protein supply is not only essential during periods of growth but also during pregnancy. Dunlap and Dickson (1955) found that ewes had a decreased concentration of albumin during the first half of pregnancy but the levels returned to near normal at term. They related this to its requisite usage as protein precursors. They also found that in the latter half of pregnancy, there was a decrease in globulin concentration and this was thought to be due to the production of globulin - rich colostrum. Similar observation was made by Wright et al., (1962) in sheep, and in cows by Larson (1958) who found a decrease in the concentration of total protein and especially beta and gamma globulins.

Hart and Miller (1937) reported a low reproductive performance for sheep on a low protein diet. Similar findings have been reported in laboratory animals (Guilbert and Gross, 1932). This is said to be due in part, to the lack of leuteinizing hormone and the general physiological stress due to protein deficiency.

The nutritional status of a developing fetus is to a great extent dependent on the mother's nutrition (Smith, 1947; Bergner and Susser, 1970). Malnutrition in late pregnancy has been suggested as a possible contributory factor to neonatal mortality among beef calves (Delong et al., 1979). Circumstances of adverse nutrition have yielded premature and small for date babies in humans (Antonov, 1947). Ewing (1959) also reported that cows fed protein supplement gave birth to heavier calves than those not fed protein supplement.

Apart from the use of protein for growth, Blaxter and Martin (1962) reported that any diet given to ruminants with more protein above the needs for maintainance and growth was converted to energy, though they pointed out that protein is not utilized efficiently for energy by ruminants.

Nutrition and the Blood Profile

Hewett (1974) indicated that there is an association between nutrition and the blood profile. Borderline signs of anaemia in heifers fed low levels of dietary protein has been reported by Bedrak et al., (1964). They found 30% reduction in hemoglobin on consumption of 0.08 kg crude protein.

Greig and Boyne (1956) showed that plane of nutrition affected hemoglobin levels. Simple correllation did not indicate a consistent association of hemoglobin concentration and growth traits. However, Hewett (1974) reported a significant correllation between dietary components, hemoglobin, and packed cell volume. Saror and Cole (1975) reported that Zebu cattle under native husbandry practices had lower packed cell volume and h emoglobin levels than those kept in temperate countries. This was attributed to lower nutritional status and stress. It has been reported that protein deprivation lowers serum protein levels in cattle (Bedrak et al., 1964; Little and Manston, 1972; Blowey, 1973). Serum albumin levels are also affected by level of nutrition. Whiting et al., (1951) reported that it is specifically the albumin fraction that is altered during conditions of dietary insufficiency. He found no significant difference in the various globulin fractions of sheep. Cooper et al., (1974) reported a marked decrease in the various serum protein fractions in mice fed a protein-dificient diet. Canon (1942) first reported depression of gamma globulin levels in malnutrition.

Saror and Schillhorn van Veen (1977) and Oduye (1977) reported lower haematological values in clinically normal Nigerian sheep than the temperate breed of sheep. This difference was attributed to lower nutritional status in the Nigerian sheep. Another report by Anosa (1977) indicated that helminthiasis could also be a significant source of lowered haematological values in the Nigerian sheep.

Structure and Function of the Immune System

The immune system is extremely complex, and is currently under active investigation on cellular and molecular levels. Scientists are just beginning to understand how the different components of the system interrelate. In the body, there are three different classes of immunologically active white blood cells: granulocytes, monocytes, and lymphocytes. These cells are derived from a common hematopoietic stem cell found in the bone marrow of the adult. Granulocytes provide for primary host defense against pyogenic invasive organisms and are responsible for the acute inflammatory response. Monocytes and their progeny-the macrophages-are responsible for the secondary line of defence in phagocytosis, known as the delayed

or chronic inflammatory reaction. Monocytes and macrophages, however, are only as soldiers acting under orders from the third set of immunologically active cells, the lymphocytes (Good and Jose, 1975).

There are two major arms of the lymphoid system, the T-cell component which is thymus dependent and the B-cell component which is thymus independent and derived from the bone marrow in mammals. Recently a third, the K-cell a special form of T-cells has been identitied. In birds, the function of the B-cell component is dependent on a special organ called the bursa of fabricius. (Good and Jose, 1975). Recent evidence from Owen et al., (1974) indicates that one site which may exercise the bursal function in mammals is the fetal liver.

B-cells make up to 5-12% of the blood lymphocytes. Characteristically, they are also found in the germinal centres and medullary cords of the lymphnode. In the spleen, they are at the chief site of splenic antibody production, which is in the lymphoid follicles of the white pulp and red pulp cords. Approximately 50% of the total lymphocyte population made up of B-cells are found also in the tonsils and peyers patches of the gut.

As a group, B-lymphocytes are more sessile than the T-cells (Cooper et al., 1970).

In charge of 'humoral immunity' and progenitor of plasma cells, B-cells synthesize, and in their fully differentiated form, secrete immunoglobulins and specific antibodies which provide the individual with a primary defence against high grade encapsulated bacterial pathogens. Their products-antibodies-serve to detoxify certain proteins, polypeptides, and other toxins. They neutralize viruses, and interfere with absorption of foreign protein and antigenic material from the respiratory tract and gut (Good et al., 1972).

Scanning electron microscopy photos show that B-cells have a more villous surface than that of the generally smooth looking T-cell. On their surface B-lymphocytes carry immunoglobulin molecules which presumably function as specific antigen receptors. The majority of the B-cells circulating in the blood carry both IgM and IgD on their surfaces. A smaller number of the circulating cells may carry either IgE, IgA or IgG. B-cells may also have an Fc surface receptor, which is capable of combining with the Fc portion of an immunolobulin molecule, and a C_3 receptor, which can combine with the third component of complement (Good et al., 1972).

The T-cells which are responsible for "cell-mediated immunity" make up the vast majority (75-90%) of blood lymphocytes. They can also be found in the deep cortical areas of the lymph node and in the perivascular areas of the splenic white pulp. Like the B-cells, they are in the tonsils and the peyers patches of the gut. Containing both short and long lived components, T-cells as a population are very mobile, and can be thought of as a rentable flying squadron of cells (Cooper and Good, 1971).

It is the T-cells that provide a major specific component in the defence against facultative intra-cellular bacterial pathogens, fungi and many viruses. They function to initiate solid tissue allograft rejection, delayed hypersensitivity reaction and graft versus host reaction. They also provide lymphokines (Jose and Good, 1973b).

The T and B-cells working together provide the full expression of immunity. While different in many respects, they both achieve their goals by calling on fundamental effector processes, including phagocytosis, activation of complement alterations of blood flow through changes in vascular reactivity, inflammation, and blood

coagulation (Copper and Lawton, 1974).

Malnutrition and Disease

There appears to be a relationship between nutrition and infection in animals (Alexander and Meleney, 1935; Miner, 1955; Scrimshaw et al., 1959; Gordon et al., 1964; Jose and Good, 1973a; Cooper et al., 1974; Fernandes et al., 1976). Faulty nutrition very obviously impairs the productivity of animals to the extent that their health is affected and sometimes recognisable disease ensues.

Some work has been done to describe in clinical, biochemical and pathological terms the effects of removing a nutrient from an animal's diet. As a result, there is usually thought to be a clear association of deficiency of a nutrient with a specific disease syndrome. As examples, xerophthalmia has been associated with vitamin A deficiency, microoytic anaemia with iron deficiency, myopathy with tocopherol and selenuim deficiencies and so forth. However, in practice there are nutrient interactions and species differences and this makes it difficult for such generalizations (Blaxter, 1979).

Jose et al. (1970) observed that malnourished Australian aboriginal children frequently exhibited rheumatic heart disease. Rhoads and Alexander (1955) had reported on the increased incidence of post-operative infection in surgical patients with hypoproteinemia.

Malnutrition has generally been noted to act synergistically with infection by most classes of bacteria particularly the pyogenic pathogens, but occasional antagonistic effects occurred with myco-bacterium tuberculosis, pasteurella, and some salmonellae (Scrimshaw, 1966). Striking decreases in the incidence of spontaneous tumors and marked slowing in local and metastatic spread of established malignant neoplasms in chronically malnourished animals was first reported by Rous (1914). These findings have been confirmed for many tumour types in rodents and cattle when their intake of either protein or calorie is reduced (Jose and Good, 1971).

Effects of Malnutrition on the Immune Response

Field studies and controlled laboratory experiments have shown that nutrition has a profound effect on the immune system (Dennis et al., 1974; Cooper et al., 1974; Robin and Hazel, 1975;

Fernandez et al., 1976; Page-Faulk et al., 1979). The interactions of nutrition and immune responses are complex and are further compounded by . . . environmental and stress conditions. In nature, when nutrient deficiencies do occur, they are usually multiple and are found in association with varying degrees of parasitism and contamination in the environment (Sheffy and Williams, 1982).

The essential influence of one nutrient deficiency on immunity is very variable with too many unknown factors at play (Good and Jose, 1975).

It has been reported that a diet relatively high in fat and low in protein fostered greater body weight, better reproduction but earlier onset of and more severe hemolytic anemia in NZb mice - (NZb mice a strain with a marked predilection to develop and die of hemolytic anemia (Good et al., 1976). They also reported that animals on the higher fat fiet had more leukocytosis and reticulocytosis, an increased severity of coombs positivity, and more autoantibodies (especially (IgG and IgA) on red cell surfaces than did mice on the lower fat diet. On the other hand, a diet relatively low in fat and high in protein brought about opposite results i.e., lower weight and poorer reproductive success, but later onset of and less

severe hemolytic anemia. With the exception of vitamin E and B_6, vitamin deficiencies more consistently influence humoral immunity than cell-mediated immunity (Sheffy and Williams, 1982).

Jose et al. (1973) reported that in guinea pigs fed 3% protein diets over an 8 week period, both T-cell and B-cell mediated immune functions were eliminated. Also Good et al. (1978) reported that a group of mice fed 8%, 6% and 0% protein (27% considered normal) intake for fourteen days revealed increased antibody response and increased plaque forming cells in proportion to the dietary deficiency. It has been reported that diets which induce chronic protein deprivation (CPD) depress function of the B-cell antibody response while enhancing many aspects of the T-cell system (Cooper et al., 1974). Also mice chronically deprived of protein showed a 30% decrease in weight gain and a marked decrease in total body protein, albumin, $alfa_1$ $alfa_2$, and gamma globulins. Hematocrit was also decreased. This was explained as been due to the fact that the body was lacking in essential amino acid needed for the manufacture of protein. The authors reported that low protein-low calorie diets are even more effective in depressing formation of blocking antibody.

It has been reported that certain individuals suffering from protein malnutrition are not able to make circulating antibodies in response to certain bacterial and viral antigens (Brown and Katz, 1966). This capacity is restored when proteins are incorporated again into the diet (Mathews et al., 1972). The degree of restoration has been correllated with the amount of protein supplementation (Reddy and Srikantta, 1964). The primary immune response to foreign antigen also reflects the degree of malnutrition (Gell, 1940).

Work done with the bovine species reveal that cows on low protein diet had a significantly lower antibody titer than those on the high protein diet when they were challenged with a formalin killed Salmonella pullorum antigen (Delong et al., 1979).

Protein deficient mice have showed increased responses to phytohemagglutinin and rejected skin allografts more rapidly than normally nourished controls while showing striking depression of splenic plaque forming cells and antibody synthesis to sheep red cells (Cooper et al., 1970). Acute protein deprivation resulted in increased antibody production and chronic protein deprivation resulted in lower antibody response (Fernandez et al., 1976).

Nutritional and infectious processes altering immune response mechanism in the laboratory rodent would also be expected to affect such responses in economic classes of livestock. Certainly we should be aware of such possibilities and give them consideration in the development and application of total health programs for farm animals. But it should be understood that laboratory conditions cannot be translated directly to field conditions with the objective of application to the more economic classes of livestock. Therefore it will be necessary to do more work in the area of examining various feeding levels as practiced in Nigeria under the different management systems as they affect the immune status.

Chapter II

DIETARY PROTEIN: EFFECT ON SOME BLOOD PARAMETERS AND WEIGHT CHANGES IN YANKASA EWES

INTRODUCTION

Monitoring hematological parameters of animals can provide useful information on their current health status. Some workers have reported that nutrition causes variations in some blood values. Greig and Boyne (1956) observed that calves on a higher plane of nutrition had higher hemoglobin levels and Hewett (1974) showed that silage feeding was a significant source of increases in the packed cell volume of cattle. Also Esievo and Moore (1979) associated low PCV with low nutrition in dairy cattle. Hassan and Hoellier (1966) noticed low blood values in sheep in the sudan during the dry season when pasture was low in nutrients as compared to other times.

There is very scanty information on the effects of nutrition on hematological parameters of sheep in Nigeria. Interestingly Luitingh (1962) correllated certain blood constituents and rate of weight gain and since live weight gain is an important factor in sheep rearing in Nigeria (because they are reared primarily for meat) this work was therefore designed to examine the effects of different planes of crude protein on some blood parameters and weight changes in the Yankasa sheep.

MATERIALS AND METHODS

Experimental Animals

Eighteen Yankasa ewes aged between six and nine months were obtained. The animals were preconditioned for three months during which treatment included deworming with anthelmintic (Thibendazole)* on arrival, daily rectal temperatures, examination of blood smears taken every week from the animals and those that had blood parasites were treated according to the parasite present. Water and mineral salt lick* were provided ad lib.

Feeding

The animals were randomly divided into three groups of six each. Group one was fed a ration containing 15% crude protein (high level), Group two was fed 7.5% (medium level) and Group three was fed 4% (low level) (Tables 1 and 2). Water and mineral salt lick** were provided ad lib.

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*Obtained from Merck Sharp and Dohme, England.

**Pfizer Mineral Salt Block - Composition; Cal - 8.5%; phosph - 4%; Mg - 108.2 PPM; Manganese - 64.9 PPM; Cooper - 86.5 PPM; Iodine, 5.4 PPM.
21.6 PPM; Zinc - 21.6 PPM; Iodine, 5.4 PPM.

Blood Sampling

Blood samples were collected monthly from all animals at 8.00 a.m. before feeding. Blood collection was by jugular venapuncture into vacutainers containing disodium salt of ethylene diaminetetracetic acid (EDTA) as anticoagulant. Hemoglobin (HB) packed cell volume (PCV), white blood cell count (WBC) where determined using routine laboratory procedures (Coles, 1967; Saror and Coles, 1973). All animals were weighed once fortnightly.

Analysis of Data

Data was analysed using statistical package for the social sciences version 6.50. One-way analysis of variance (ANOVA)* was used to test for effects of different dietary protein levels on various parameters. 5% was chosen as the significant levels.

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*As developed at Vogelback computing Centre, North-western University, April, 1976.

RESULTS

Packed Cell Volume

Table 3 shows the initial and final means of PCV in the study. The final mean PCV values were significantly different ($P<0.05$) in all three dietary groups. With the high, medium and low dietary protein groups having mean values of 34 ± 1.0; 29.5 ± 2.3; 24 ± 2.5 respectively. Table 4 and Fig. I show that the high protein group had increasing PCV values with a fall only in the third and sixth month. The highest value was observed at the 9th month of the study.

Hemoglobin

Table 3 shows the initial and final means of hemoglobin concentration in the study. The different dietary protein levels had no statistically significant effect on hemoglobin concentration during the experimental period. However, values obtained at various sampling periods were highest for the high protein group animals. (Table 5). Fig. 2 shows similar curves for all the experimental groups with the high protein group peaking at the sixth month.

White Blood Cells

The different levels of dietary protein had no significant effect on the total leukocyte, monocytes, eosinophils and lymphocyte counts (Tables 3, 6, 7 and 8). However, at the end of the experiment, the animals on the high dietary protein had a significantly ($P<0.05$) lower mean absolute neutrophil count of $2,620\pm250$/ml as compared with mean counts of $3,400\pm800$/ml and $3,500\pm405$/ml for the low and medium dietary protein groups respectively (Tables 3 and 9).

Weight Changes

The average daily weight gains were 103.7 ± 0.4 grams, 81.5 ± 0.6 grams and 53.7 ± 1.0 grams for the high, medium and low protein groups respectively and these values were significantly different ($P<0.05$) from one another (Table 3).

DISCUSSION

Results obtained from this work showed that PCV vary with level of nutrition. With the animals on the high protein diet having values that agree with those reported in clinically normal

sheep (Saror and Schillhorn van Veen, 1977). A mild degree of anaemia was observed in the group of animals fed the low protein diet. Anaemia has been observed in CPI* mice and in protein deficient humans (Cooper et al., 1974; Viteri et al., 1964). It is possible that the same pathogenetic mechanism that caused the human and mice anaemia could be said to be responsible for the anaemia observed in our experimental animals. Also the final mean haemoglobin concentration was highest for the animals in the high protein group and lowest for the animals in the low protein group. Though the differences were not statistically significant, this graded effect might be attributed to nutritional influences. Similar observations were made by Whipple and Maden (1944) and Yamatoto (1951) who showed that protein deficiency deprives hematopoietic organs of amino acids necessary for cell formation and hemoglobin production.

The total leukocytes, lymphocytes and eosinophils did not seem to vary with the level of dietary protein and agrees with the findings of Bedrak et al. (1957) who observed no consistent association between level of protein intake and

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*CPI - Mice - mice fed a protein deficient diet.

variations in leukocyte counts. However, the neutrophils differed significantly with diet. With the animals on the low protein diet having the highest value. This may indicate that the poorly fed animals were responding to an infection since neutrophilia is a manifestation of bacterial infection.

The animals on the high protein diet had higher daily weight gains than those on medium and low. The poorer daily weight gains of the medium and low protein groups might suggest an inhibition of cellular proliferation evident in the reduction of body growth. This study therefore showed that normal haematological values and weight gains were observed for the animals fed the 15% crude protein supplement and that adverse effects of low dietary protein became evident at the 7.5% and 4% crude protein supplementation.

Chapter III

DIETARY PROTEIN: EFFECT ON SERUM PROTEIN FRACTIONS AND IMMUNE RESPONSE IN YANKASA EWES

INTRODUCTION

Over 60% of the total population of sheep in Nigeria are reared in the Sudan and Sahel zones, where availability of pasture varies with the season. This problem is further compounded by the fact that a greater proportion of these animals are reared by nomadic herdsmen, who husband their animals in the age old traditional methods without any innovations. They depend largely on grazing pasture with little or no supplements. Thus for half of the year (during the hot harmattan period) these animals are exposed to periods of scarcity and malnutrition especially protein malnutrition.

Various studies have indicated an association between malnutrition and an impaired immune response (Gordon et al., 1964; Jose et al., 1973; Good et al., 1976; Fernandez et al., 1976). Of the six major nutrients, carbohydrate, fat, protein, vitamins, minerals and water, protein has limiting effect on the immune response (Cooper et al., 1974). But these observations were made under controlled laboratory conditions and cannot easily be translated to field conditions with the objective of application to the economic classes of livestock.

This study was therefore designed to examine the effects of varying levels of crude protein on serum protein fractions and immune response in ewes.

MATERIALS AND METHODS

Eighteen Yankasa ewe lambs were obtained, preconditioned and fed for the experiment as previously described in Chapter Two.

Blood samples were collected from all animals monthly, normally at 8.00 a.m. before feeding. Blood collection was by jugular venapuncture. Clotted blood was centrifuged, serum decanted and stored at -20°C until analysed.

Serum total protein was determined by the Biuret method. Serum electrophoresis was carried out on cellulose acetate strips using Beckman B-2 buffer with ionic strength of 0.075 at pH 8.6 and dried. The dried electrophoretic strips were fed into the Beckman densitometer and traces for the different serum protein fractions were obtained.

Six months after the initiation of the dietary regimen, the animals were all vaccinated with formalin killed suspension of Salmonella gallinarum*.

*Salmonella gallinarum stock antigen - obtained from the Nigerian Veterinary Research Institute, Vom.

Each animal received about 6×10^6 organisms intraperitoneally. A second equal dose was given through the same route two weeks later. Serum samples were collected preinjection and at 24 hours, 72 hours at two weeks, three weeks and eight weeks following the second dose. Bacterial tube agglutination test was carried out on these serum samples using the salmonella polyvalent antigen**. Data was analysed using statistical package for the social sciences version 6.50. One-way analysis of variance (ANOVA)*** was used to test for the effects of the different protein levels on the various parameters. 5% was chosen as the significant levels.

RESULTS

Total Serum Protein

Table 1 shows results obtained from the effects of different levels of dietary protein on total serum protein. There was a significant effect of feed on the total serum protein level ($P<0.05$ (Table 10 and 11). The animals on the high protein

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**Salmonella polyvalent antigen was obtained from Salsbury Laboratories, Charles City, Iowa.

***ANOVA. As developed at Vogelback Computing Centre, North-western University, 1976.

diet had significantly higher final mean value (8.09±.08) than those on medium (6.41±0.23) and low (6.41±.02). Fig. 4 indicates a fall in the total serum protein level for the animals on the low protein diet at the second month of the experiment which remained almost at the same level for the rest of the experimental period.

Albumin, $Alfa_1$, $Alfa_2$, Beta and Total Globulins

The different dietary protein levels had no significant effect on the albumin, $alfa_1$, $alfa_2$ and beta globulins (Tables 14, 15, 16 and 17). Fig. 5-10 show the pattern obtained for these different fractions. Towards the end of the experiment the pattern observed for albumin was similar for the animals in the medium and low protein groups (Fig. 5).

Gamma Globulins

The different dietary protein levels had a significant effect ($P<0.05$) on the gamma globulin levels (Tables 12 and 18). The mean value for the animals in the high protein group was 3.08±0.02 and this was significantly different from those on

medium and low protein diet which was 2.51 and 1.75±0.5 respectively (Table 12). Fig. 7 shows the pattern obtained for the gamma globulins. The animals in the low protein group showed similar changes with those on high although the concentration obtained differed. The high protein group peaked at 1.20gm/dl.

Bacterial Tube Agglutination Test

The animals on the high protein diet had significantly different antibody titers at two weeks post innoculation. At three weeks post innoculation significantly different titers were observed between the experimental groups. At eight weeks post innoculation only the high protein group again had significantly different antibody titer (Table 11).

DISCUSSION

Results in this study showed that different levels of dietary protein cause variations in the total serum protein levels. The animals on the low protein diet had significantly lower serum protein value as compared to those on high. Similar findings

have been reported in Dorset sheep by Klosterman et al. (1950) and Wright et al. (1962). While this finding may not indicate the sites of breakdown or failure in complex immune mechanisms it may be an indication of a lower total defensive capacity of the animals in the low dietary protein group as compared to those on high.

Though there was no significant effect of the different crude protein levels on the serum albumin concentrations, the low and medium protein groups had lower values at variouse sampling periods (Tables 5, 6 and Fig. 7). This agrees with Klosterman et al. (1950) who indicated that it is specifically the albumin fraction that is altered during conditions of dietary insufficiency, because albumin serves as a major source of essential amino acid required as protein precursors.

The gamma globulin fraction were significantly affected by the different diets. The consistently lower values obtained for the low and medium protein groups is in agreement with the findings of Canon (1952) who reported a reduction of gamma globulins in mice fed a protein deficient diet as compared to those on normal diet. The other serum globulin fractions were essentially unaffected by the different

diets.

The animals on the high protein diet had significantly higher antibody titer to the antigen as compared to those on medium and low at various sampling points. This agrees with the findings of other workers who have shown that protein deprivation results in decreased antibody production (Jose et al., 1973; Page-Faulk et al., 1974; Good et al., 1976). This finding may explain at least in part the reduced gamma globulin in the low protein group.

It is therefore concluded from this work that dietary crude protein levels affects levels of total serum proteins especially the albumin and gamma globulin fractions and that antibody production is compromised in circumstances where the dietary protein is low.

Chapter IV

SUMMARY AND CONCLUSIONS

Malnutrition in the Nigerian livestock population has been mentioned by various workers but its effect on body patho-physiological processes has not been fully quantitated.

This study was designed with the recognition that our best would only be an approximation towards mimicking the nutritional situation in animals reared under the traditional system of husbandry which heavily depends on range grazing and those that are reared under semi-intensive method in the few government and individual farms. It was to answer the question on the effects of protein malnutrition (using local feeding ingredients for our experiment ration) on body weight, some blood parameters and the immune response.

The findings show that better performance, better blood picture and stronger immune response was observed in the animals fed the 15% crude protein as compared to those on the 7.5% and 4.% crude protein diet.

Even in the very developed nations where great strides have been taken in farm management practices, there are times when the animals are exposed to periods of scarcity and the animals performance and immune response are temporarily compromised.

Nigeria being a country that is still plagued with various infectious diseases as far as livestock is concerned, there is every need to ensure that our livestock are immunologically capable to respond to the various disease assaults. One of the ways of ensuring this is to always seek to improve the nutritional status of the animals.

Most of the national herds depend heavily on range grazing alone (pasture during the dry season is said to contain less than 4% crude protein and about 6% during the rains). As evidenced from this work, animals fed protein supplements above these levels had better performance and stronger immune responses. So in order for the green revolution programme and other livestock programmes currently going on in the country to succeed, emphasis should be geared more towards making animals feeds and supplements available to the local farmer.

This work has only touched the top of the iceberg. The immune system is very complex and scientists all over the world are just beginning to understand the complex interrelationship between malnutrition and the immune system. The following areas are recommended as possible areas for future research:

1. effects of nutrition on cellular aspects of immunity;
2. the adverse effects of malnutrition is not always as a result of a single nutrient deficiency but often times, there is nutrient interaction. It will therefore be necessary to research into other nutrients as they affect the immune response.
3. finally, most of the feeds used as animal feeds presently are all staple human foods. With the growing population in Nigeria, it may be necessary to research into feeds that are equally high in protein which could be used as animal feeds but which are not consumed by human population.

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Figure 1

Effects of different crude protein level on packed cell volume

Figure 2

Effects of different crude protein level on hemoglobin

Figure 3

Effects of different crude protein level on total white blood cell

Figure 4

Effects of different crude protein level on serum total protein

Figure 5

Effects of different crude protein level on serum albumin

Figure 6

Effects of different crude protein level on total serum globulin

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Figure 7

Effects of different crude protein level on Alfa$_1$ globulin

Figure 8

Effects of different crude protein level on $Alfa_2$ globulin

Figure 9

Effects of different dietary protein level on Beta globulin

Figure 10

Effects of different crude protein level on gamma globulin

Table 1: PERCENTAGE COMPOSITION OF EXPERIMENTAL RATION

	PLATE I	PLATE II	PLANE III
Concentrate mix (Guinea corn & cotton seed cake)	63.9	27.0	9.83
Andropogon Gayanus (Gamba hay)	34.4	71.3	88.5
Bone meal	0.8	0.7	0.7
Common salt	1.0	1.0	1.0
Crude Protein Level	15%	7.5%	4%

Table 2: (%) PROXIMATE COMPOSITION OF EXPERIMENTAL RATIONS

Level of Nutrition	MD	N_2	EE	CF	ASH
High Level	90.0	2.2	2.4	3.4	3.5
Medium	89.3	1.94	2.25	5.85	2.6
Low	91.3	,45	1.9	42.9	7.9

DM = Dry Matter

N_2 = Nitrogen Content

EE = Ether Extract

DF = Crude Fiber

TABLE 3: EFFECTS OF DIFFERENT LEVELS OF DIETARY PROTEIN ON SOME BLOOD PARAMETERS AND WEIGHT CHANGES IN SHEEP

Crude Protein	PCV %		Haem. gm/dl		Total WBC/ul		Number of Lymphocytes		Number of Neutrophils		Number of Eosinophils		Weight Changes in Grams	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
15%	29±1.0	34 ±1.0[a]	6.60 ±1.0	10.95 ±0.5	11358 ±2000	11358 ±1050	5770 ±1616	7600 ±400	5031 ±1168	2620 ±250[a]	568±564	1138 ±400	20015 ±500	38888.4[a] ±3522
7.5%	29.5 ±0.5	29.5 ±2.3[b]	6.70 ±2.0	9.2 ±1.0	10600 ±2500	11925 ±2000	5981 ±1233	7400 ±700	4115 ±352	3350 ±405[b]	504±177	1175 ±175	20020 ±210	34853.0[b] ±1922.0
4%	29.5 ±2.0	24 ±2.5[c]	6.90 ±2.0	8.7 ±2.0	10586 ±2750	12080 ±500	5818 ±1291	7500 ±100	4136 ±861	3400 ±800[b]	532±370	1180 ±300	20018 ±216	29791.4[c] ±2000

a, b, c. - data with different superscript within each column are statistically significantly different (P 0.05)

Table 4: MEANS ± SD FOR PACKED CELL VOLUME OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

(%)

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	29±1	29.5±0.5	29.5±2
1	29.5±2	27±1.5	23.9±7
2	30±.5	28.5±2	24±2.5
3	29±1.5	27±0.2	25±1
4	31±.2	29±2	24±4
5	32±6	30±3.5	24±.5
6	29±.8	29±4	25±1.5
7	31±1.5	29.5±2.5	24±2.5
8	32±0.5	30±1	28±1
9	34±1	29.5±2.3	24±2.5

Table 5: MEANS ± SD FOR HAEMOGLOBIN OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN (gm/dl)

Time of Sampling (in months)	LEVELS OF NUTRITION		
	High	Medium	Low
0	6.60±1	6.70±2	0.9±2
1	6.62±0.5	6.78±.4	6.94±.6
2	9.62±0.2	9.92±2.0	8.78±1
3	10.8±2	10.2±2	8.9±1
4	9.28±.4	9.1±0.4	8.18±0.7
5	9.5±.6	9.0±1	8.3±2
6	10.96±1.4	9.4±1	8.6±.3
7	10.9±2	10.3±2	8.9±1.5
8	10.86±3.4	10.4±2	8.9±3
9	10.95±.5	9.2±1	8.7±2

Table 6: MEANS ± SD FOR EOSINOPHILS OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	568±564	504±177	532±370
1	369±252	178±51	1047±857
2	312±170	225±112	593±673
3	312±171	224±111	1177±536
4	1193±197	945±305	1178±537
5	1194±196	946±302	1073±366
6	1383±557	1661±690	1073±367
7	1137±491	1150±193	1074±352
8	1230±350	1250±190	1100±250
9	1138±400	1175±200	1180±300

Table 7: MEANS ± SD FOR MONYCYTES OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	0	0	0
1	0	0	0
2	130±120	0	0
3	0	50±40	0
4	0	0	0
5	110±120	0	0
6	0	0	0
7	0	0	120±90
8	0	0	130±50
9	0	0	0

Table 8: MEANS ± SD FOR LYMPHOCYTES OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	5770±1616	5981±1233	5818±1291
1	5742±1481	6339±1301	5235±1291
2	5855±1451	6390±978	9076±873
3	6594±464	6566±632	5073±870
4	7192±789	6058±848	5203±575
5	7589±313	6058±800	6203±378
6	6057±848	6058±847	5944±1030
7	7589±300	6088±264	6046±624
8	7500±350	7008±900	7040±700
9	7600±400	7400±700	7500±100

Table 9: MEANS ± SD FOR NEUTROPHILS OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	5031.0±1168.0	4115±1352	4136±861
1	5256±1253.0	3706±1294	4450±654
2	5202.0±1306.0	3732±807	4424±976
3	3826±674	2760±741	3087±524
4	2814±520	2788±348	4424±976
5	3837±632	2760±122	3087±523
6	2814±520	2761±248	4057±447
7	2615±293	3371±467	3377±930
8	2716±200	2800±300	3300±350
9	2620±250	3750±405	3400±800

Table 10: MEANS ± SD OF TOTAL LEUCOCYTES OF EWES FED DIFFERENT LEVELS OF DIETARY PROTEIN (WBC/Ul)

Sampling period in months	LEVEL OF DIETARY PROTEIN		
	High	Medium	Low
0	11358±2000	10600±2500	10986±3740
1	11369±292	10223±3091	10731.9±2226
2	11369±302	10347±3092	10095±2422
3	10733.1±314	9450±2591	9342±1950
4	11199±310	9762.9±2501	11805±2547
5	12621±321	9959±2473	10363.0±2584
6	10254±239	9969±2979	11124±2478
7	11340±280	10609.8±3159	10497±2488
8	11446.2±276	11058.0±2384	11440±3003
9	11358±1050	11925±2000	12060±2719

Table 11: BACTERIAL TUBE AGGLUTINATION TITERS OF SHEEP FED DIFFERENT LEVELS OF CRUDE PROTEIN INOCULATED WITH SALMONELLA VACCINE

Level of (Crude) Nutrition (Protein)	Geometric Mean Titers					
	Preinoculation	24 hrs. Post Inoculation	72 hrs. Post Inoculation	2 weeks Post Inoculation	3 weeks Post Inoculation	8 weeks Post Inoculation
High (15%)	3.76 ± 1.0	3.76 ± 1	3.76 ± 0.6	16 ± 2^{a}	128 ± 2^{a}	16 ± 0.6^{a}
Medium (7.5%)	3.76 ± 0.8	3.76 ± 1	3.76 ± 1	8 ± 2^{b}	64 ± 1^{b}	12 ± 0.8^{b}
Low (4%)	3.76 ± 0.6	3.76 ± 0.4	3.76 ± 0.4	8 ± 1^{b}	29.8 ± 4^{c}	11.3 ± 1^{b}

a, b, c - Data with different superscript within each column are significantly different (P 0.05).

Table 12: EFFECTS OF DIFFERENT LEVELS OF CRUDE PROTEIN ON TOTAL SERUM PROTEIN AND SERUM PROTEIN FRACTIONS OF EWES

Crude Protein	Total serum Protein (gm/dl)		- Globulins									
			Albumia (gm/dl)		$Alfa_1$ (gm/dl)		$Alfa_2$ (gm/dl)		Beta (gm/dl)		Gamma (gm/dl)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
15%	7.41 ±0.13	8.09[a] ±0.08	3.82 ±0.02	3.62 ±0.34	0.44 ±0.20	0.56 ±0.10	0.68 ±0.01	0.98 ±0.12	0.56 ±0.01	0.91 ±0.01	2.01 ±0.02	3.08[a] ±0.02
7.5%	6.78 ±0.12	6.41[b] ±0.23	3.81 ±0.50	3.07 ±0.30	0.59 ±0.58	0.64 ±0.87	0.97 ±0.27	1.06 ±0.27	0.83 ±0.14	0.69 ±0.17	2.05 ±0.03	2.51[b] ±0.03
4%	7.17 ±0.16	6.11[b] ±0.02	3.56 ±0.04	3.53 ±0.12	0.43 ±0.02	0.56 ±0.92	0.62 ±0.98	0.96 ±0.09	0.59 ±0.01	0.69 ±0.17	2.16 ±0.22	1.75[b] ±0.5

a, b, - Data with different superscript within each colomn are statistically significantly different ($P<0.05$)

± - Standard deviation

Table 13: MEAN ± STANDARD DEVIATION FOR SERUM TOTAL PROTEIN OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN
(g/dl)

Time of Sampling (in months)	LEVEL OF DIETARY PROTEIN		
	High	Medium	Low
0	7.41±.13	6.78±.12	7.17±.16
1	8.05±.07	7.15±.01	7.03±.17
2	7.78±.02	7.15±.10	7.03±.17
3	8.50±.20	6.98±.06	6.38±.03
4	7.53±.09	7.34±.05	6.01±.14
5	7.41±.13	6.78±.12	6.56±.02
6	7.78±.02	7.40±.06	6.25±.07
7	8.18±.12	7.55±.11	6.40±.024
8	7.68±.05	7.39±.06	6.50±.01
9	8.09±.08	6.41±.23	6.41±.02

Table 14: MEANS ± STANDARD DEVIATION FOR ALBUMIN OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

(gm/dl)

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	3.82±0.02	3.81±0.40	3.56±0.04
1	3.85±0.32	3.40±0.27	3.32±0.10
2	3.73±0.32	2.70±0.29	3.31±0.20
3	3.32±0.06	3.30±0.20	2.58±0.70
4	3.31±0.01	3.32±0.30	2.66±0.70
5	3.30±0.30	3.31±0.40	3.32±0.8
6	3.74±0.34	3.48±0.67	3.01±0.11
7	4.00±.78	2.37±.78	2.91±0.37
8	3.43±0.60	2.52±0.19	3.54±0.19
9	3.62±0.34	3.07±0.30	3.52±.12

Table 15: MEANS ± STANDARD DEVIATION FOR ALFA$_1$ GLOBULIN OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

(gm/dl)

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	0.44±0.20	0.50±0.58	0.43±0.02
1	0.60±0.14	0.51±0.11	0.60±0.14
2	0.48±0.18	0.48±0.29	0.48±0.18
3	0.52±0.03	0.37±0.09	0.52±0.29
4	0.51±0.62	0.51±0.03	0.52±0.30
5	0.52±0.02	0.50±0.63	0.51±0.79
6	0.52±0.30	0.44±0.07	0.61±0.56
7	0.56±0.14	0.52±0.08	0.56±0.92
8	0.56±0.20	0.64±0.90	0.42±0.23
9	0.56±0.10	0.64±0.87	0.56±0.92

Table 17: MEANS ± STANDARD DEVIATION FOR BETA GLOBULIN OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

(gm/dl)

Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	0.46±0.01	0.63±0.14	0.59±0.01
1	0.85±0.32	0.51±0.08	0.51±0.08
2	0.81±0.13	0.60±0.14	0.60±0.14
3	0.70±0.30	0.62±0.20	0.60±0.18
4	0.71±0.20	0.63±0.62	0.59±0.02
5	0.72±0.32	0.62±0.40	0.59±0.03
6	0.66±0.20	0.64±0.31	0.64±0.31
7	0.51±0.05	0.57±0.15	0.57±0.15
8	0.71±0.20	0.55±0.06	0.55±0.06
9	0.91±0.01	0.69±0.17	0.69±0.17

Table 18: MEANS ± STANDARD DEVIATION FOR GAMMA GLOBULINS OF EWES FED DIFFERENT LEVELS OF CRUDE PROTEIN

(gm/dl)

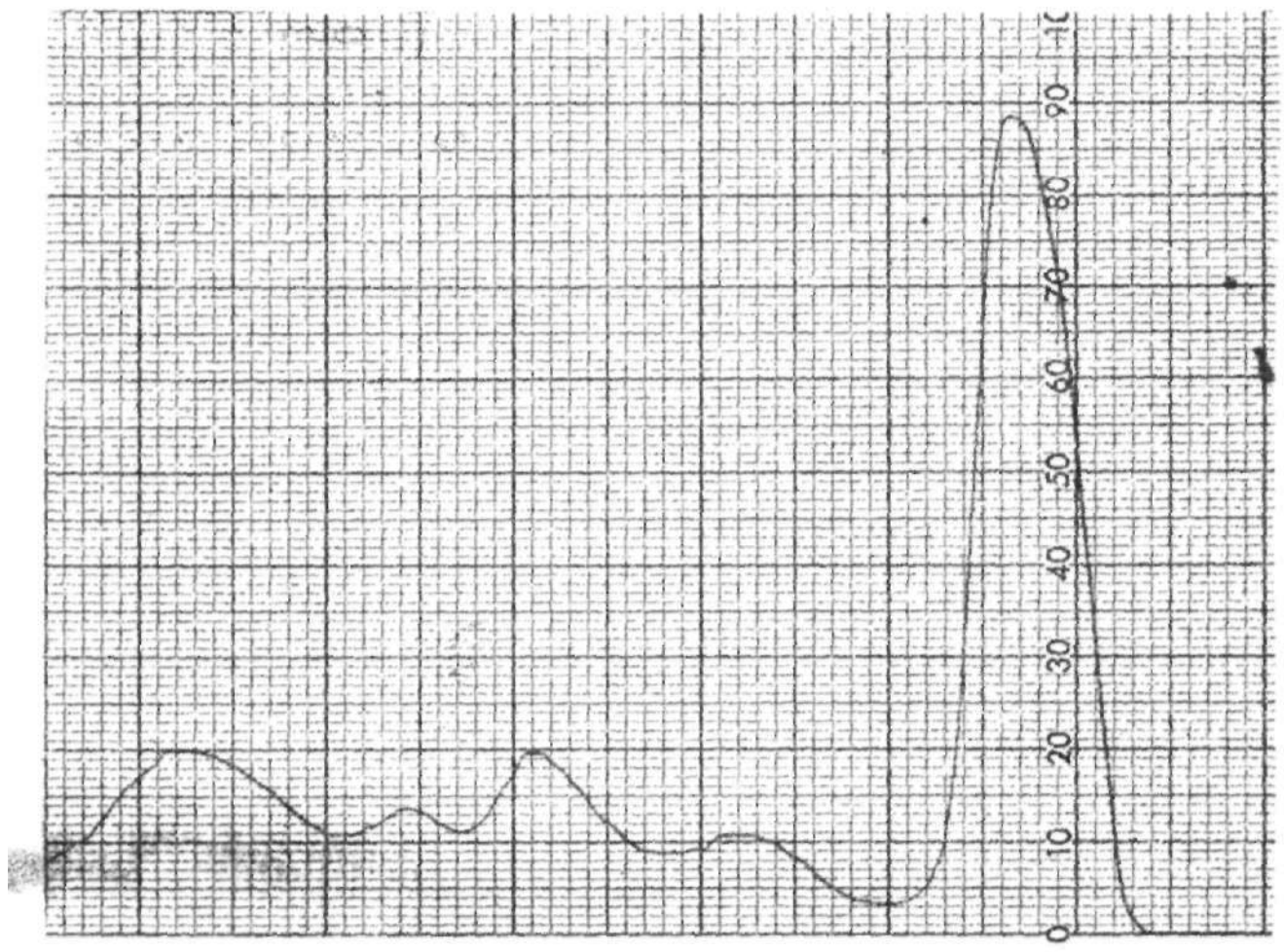
Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0 1	2.01±0.02	2.05±0.03	2.16±0.22
1 2	2.06±0.03	1.75±0.34	2.44±0.34
2 3	2.63±0.80	1.35±0.33	1.61±0.11
3 4	2.32±0.23	1.89±0.32	2.0±0.4
4 5	2.5 ±0.20	1.85±0.6	1.9±0.3
5 6	2.66±0.50	1.89±0.20	1.9±0.8
6 7	2.22±0.17	1.50±0.22	1.75±0.24
7 8	2.10±0.09	1.60±0.17	1.59±0.10
8 9	2.15±0.11	2.48±0.35	2.0 ±0.03
9	3.08±0.02	2.51±0.03	1.75±0.05

Table 19: MEANS ± SD OF TOTAL GLOBULINS OF EWES FED DIFFERENT LEVELS OF DIETARY PROTEIN (gm/dl)

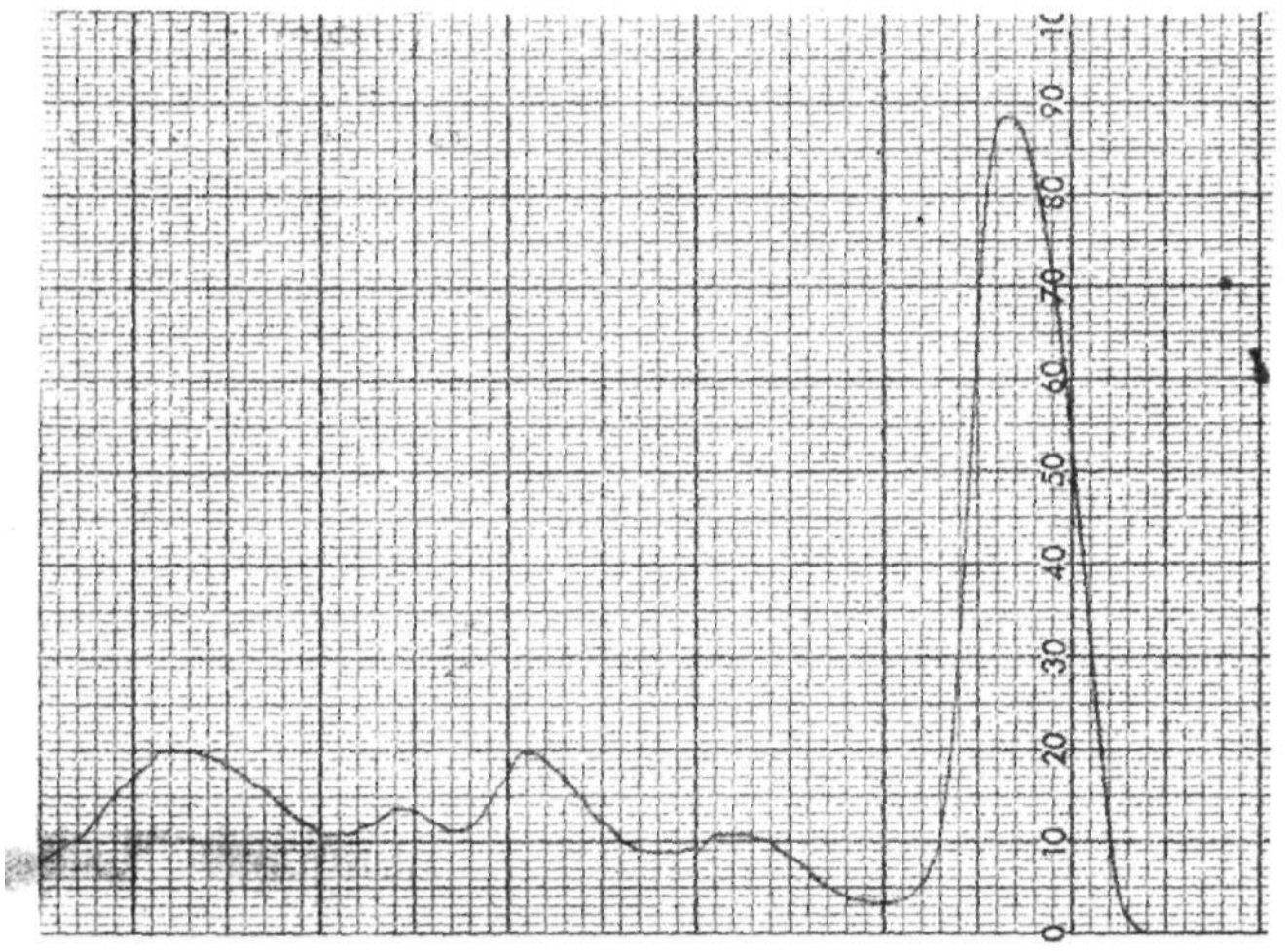
Time of Sampling (in months)	LEVEL OF NUTRITION		
	High	Medium	Low
0	3.7±.73	4.35±.67	3.8±.81
1	5.23±.69	3.78±.59	4.43±.90
2	4.64±.99	2.97±.41	3.23±.54
3	4.39±.83	3.61±.68	3.86±.70
4	4.93±.89	4.05±.61	4.13±.64
5	4.95±.97	3.93±.63	3.88±.64
6	4.46±.77	3.49±.46	3.82±.51
7	4.36±.74	3.62±.50	3.67±.44
8	4.63±.72	4.71±.89	4.08±.72
9	5.48±1.15	4.9±.88	3.96±.53

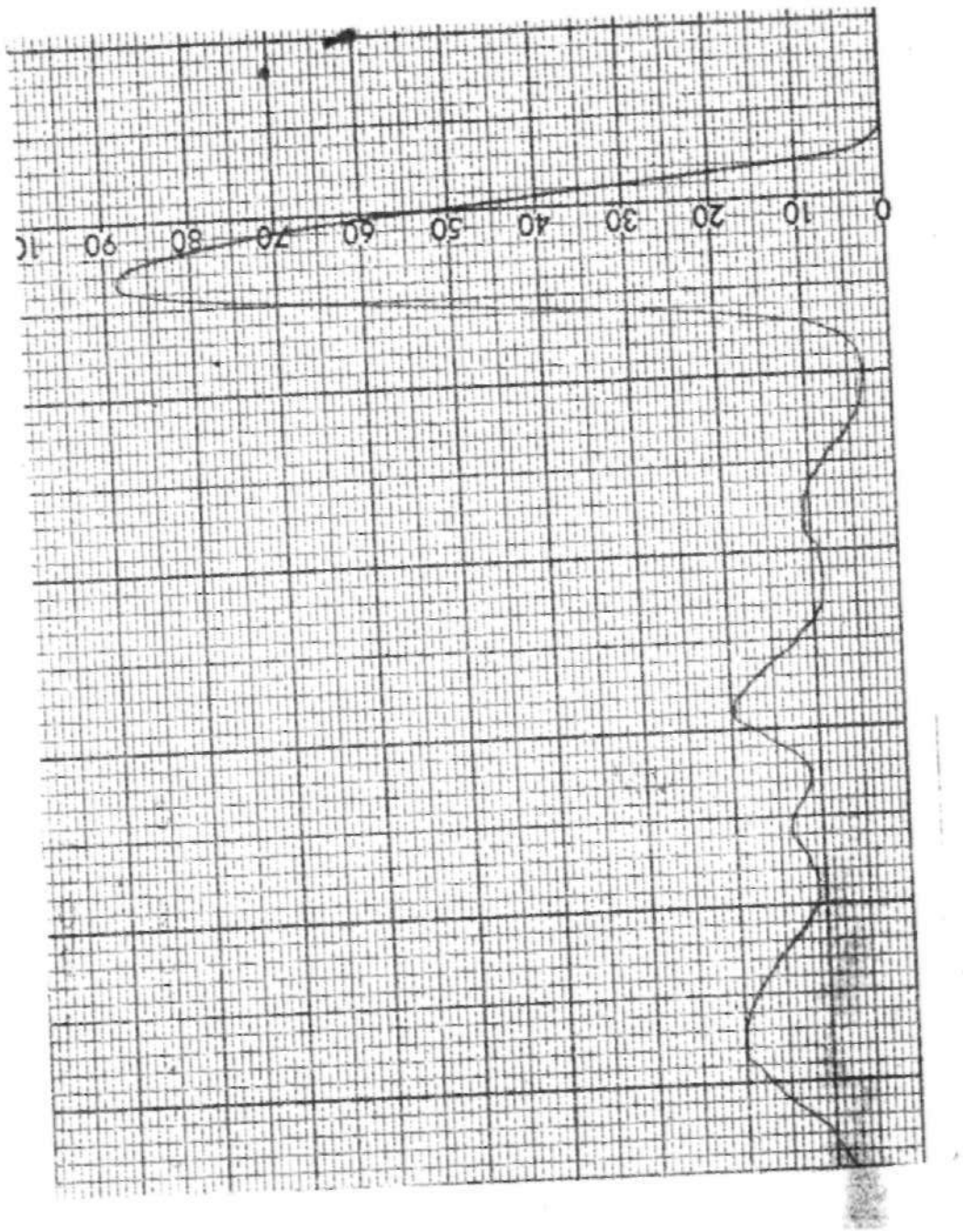
APPENDIX

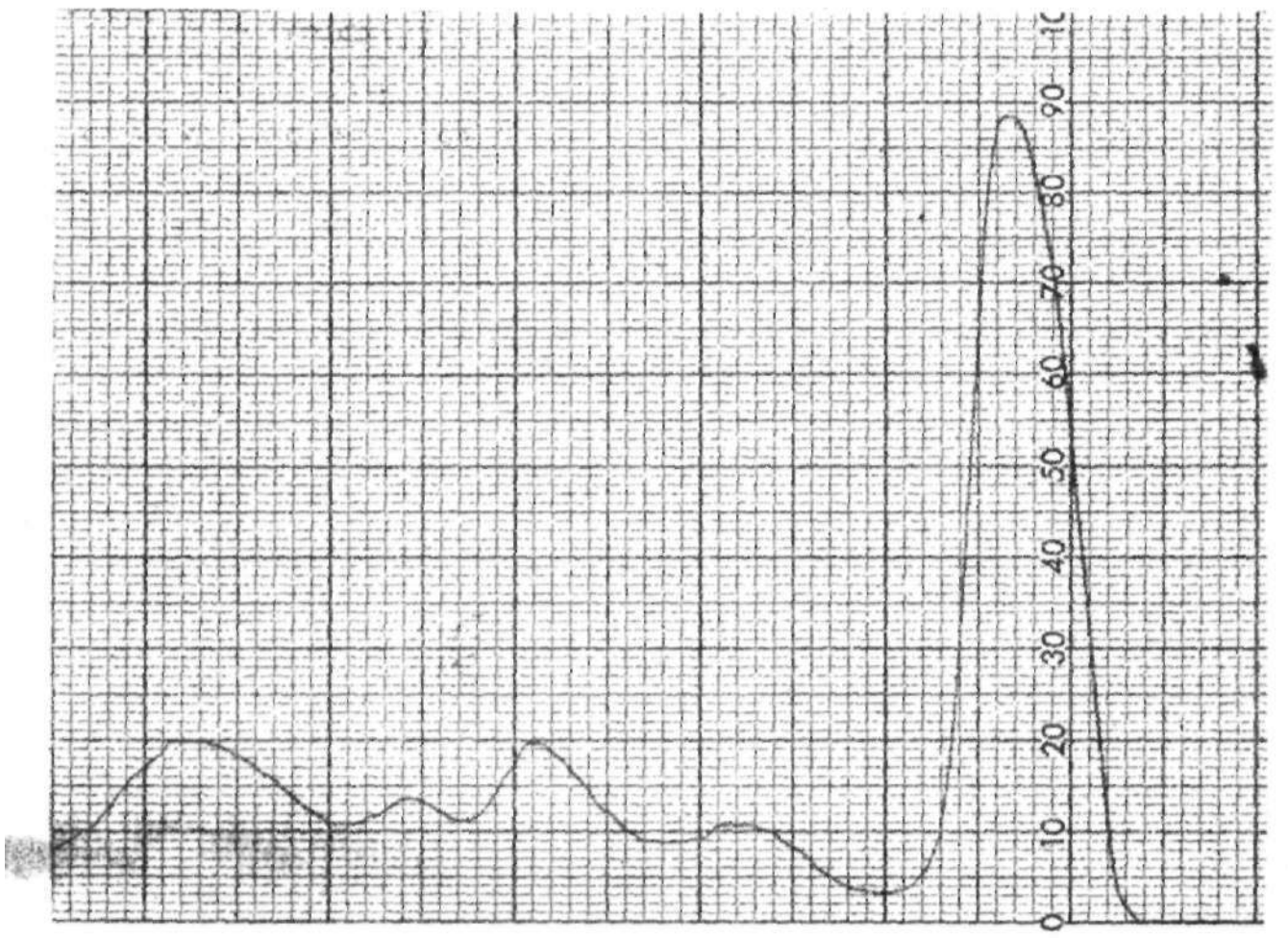
TYPICAL CELLULOSE ACETATE ELECTROPHORETIC PATTERNS OF SHEEP SERUM PROTEINS

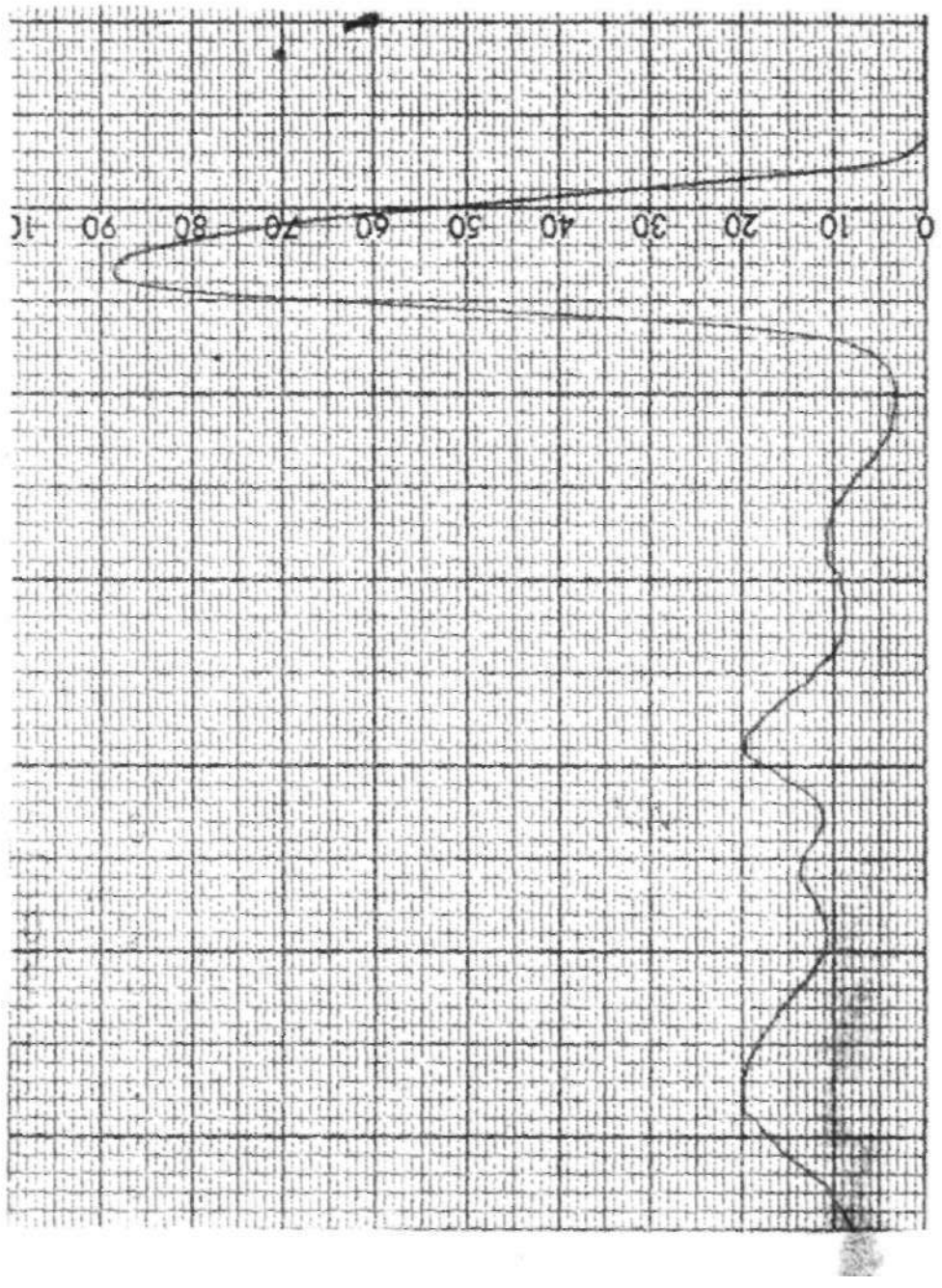

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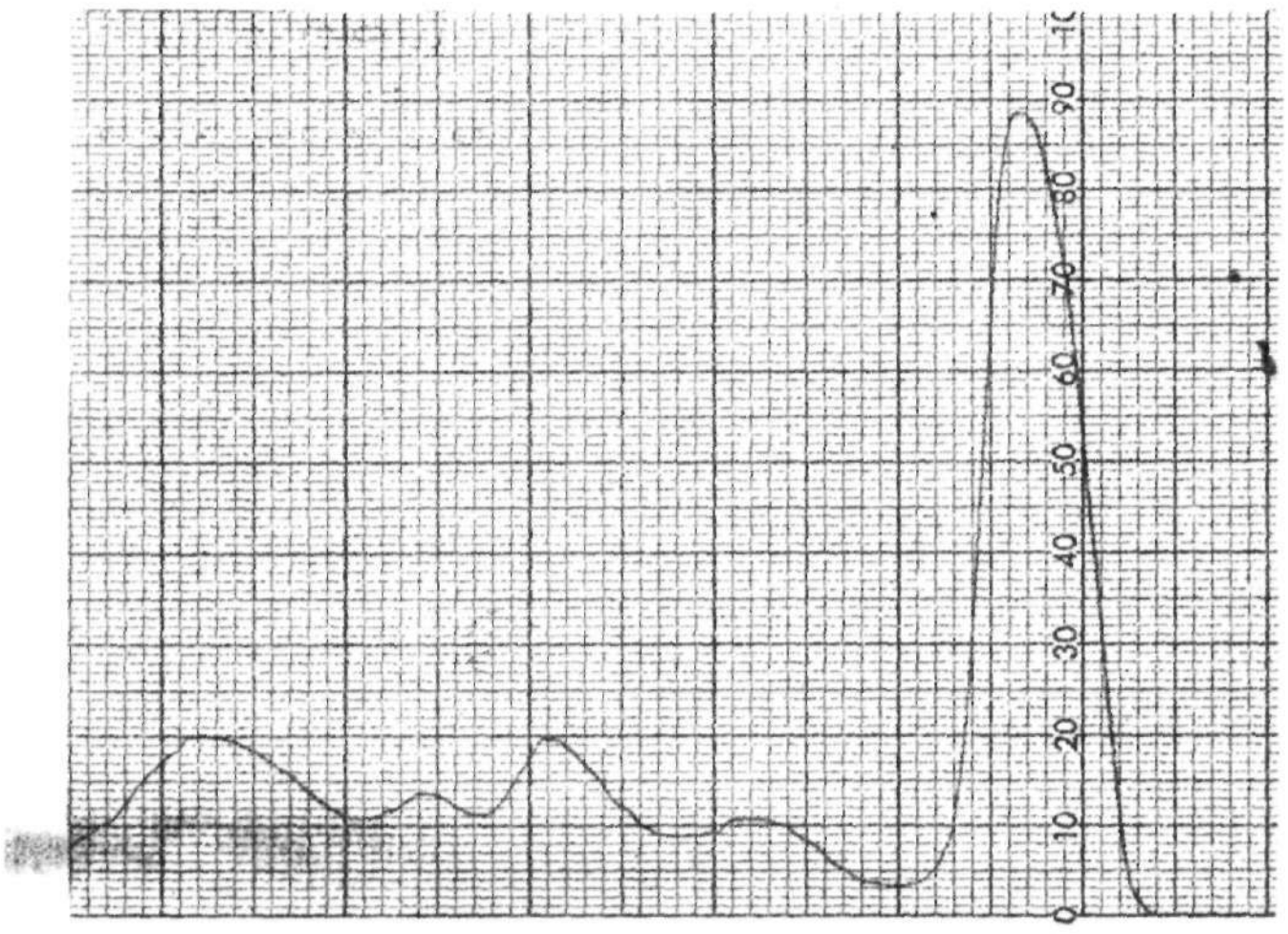

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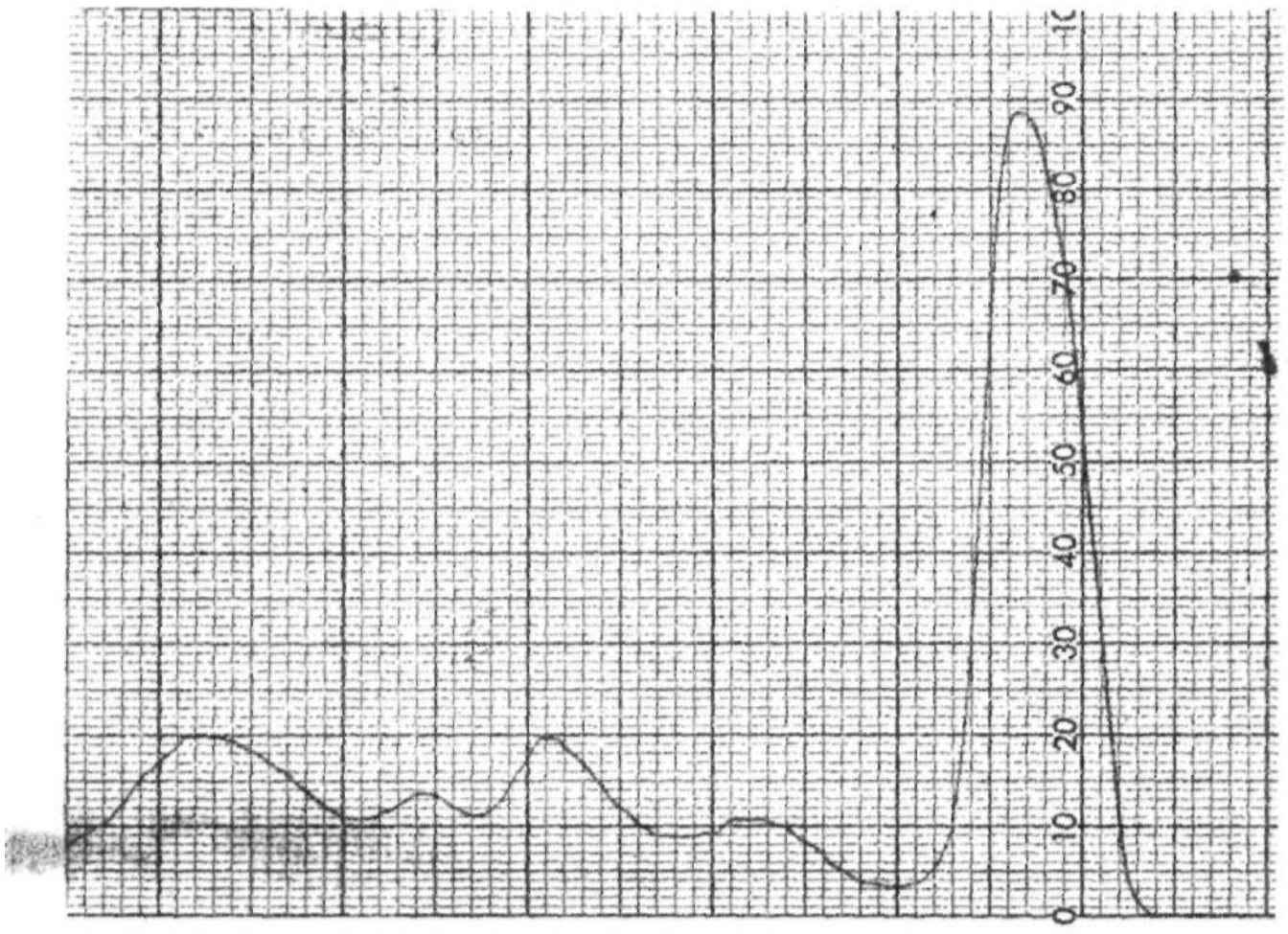

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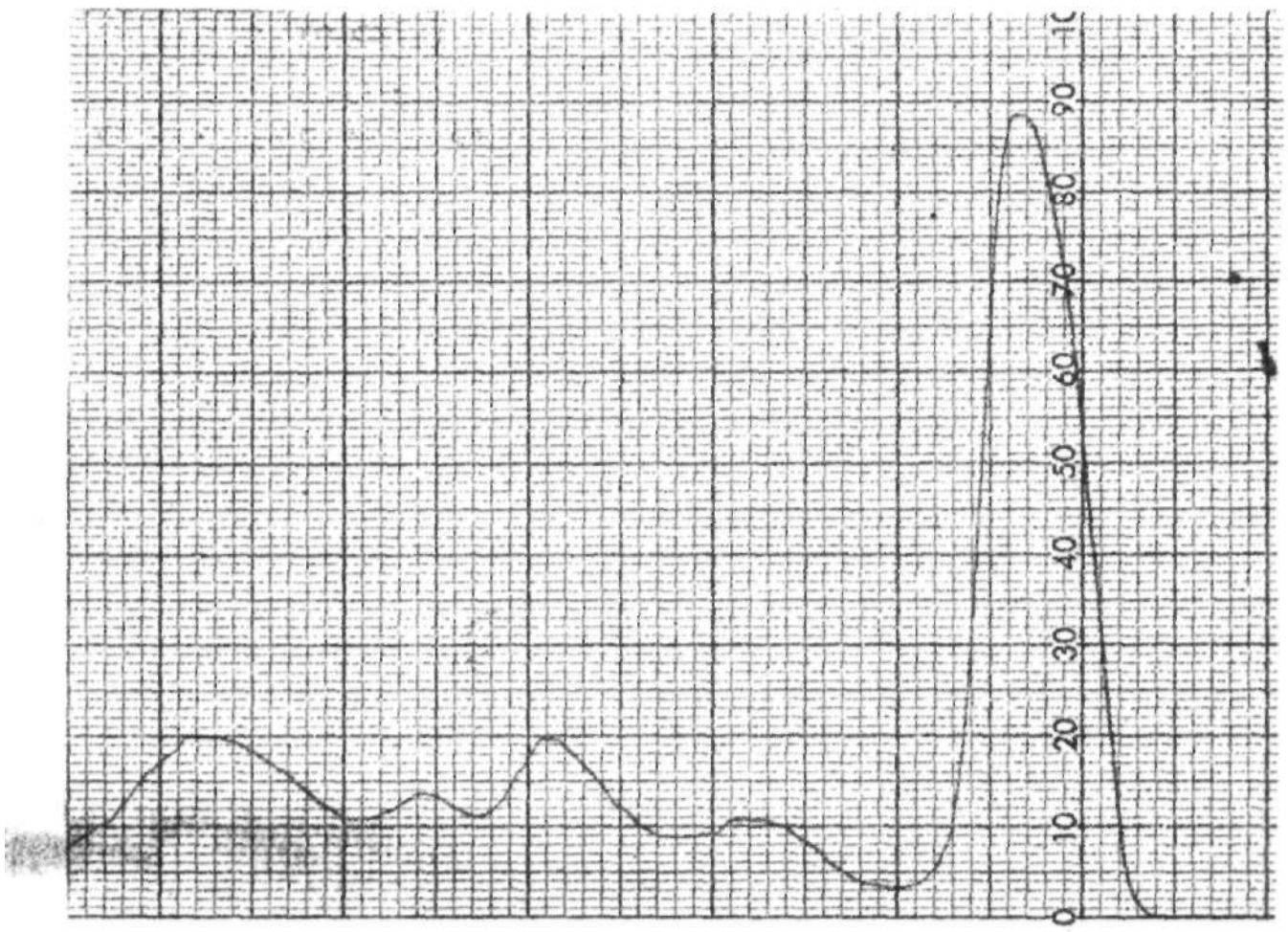

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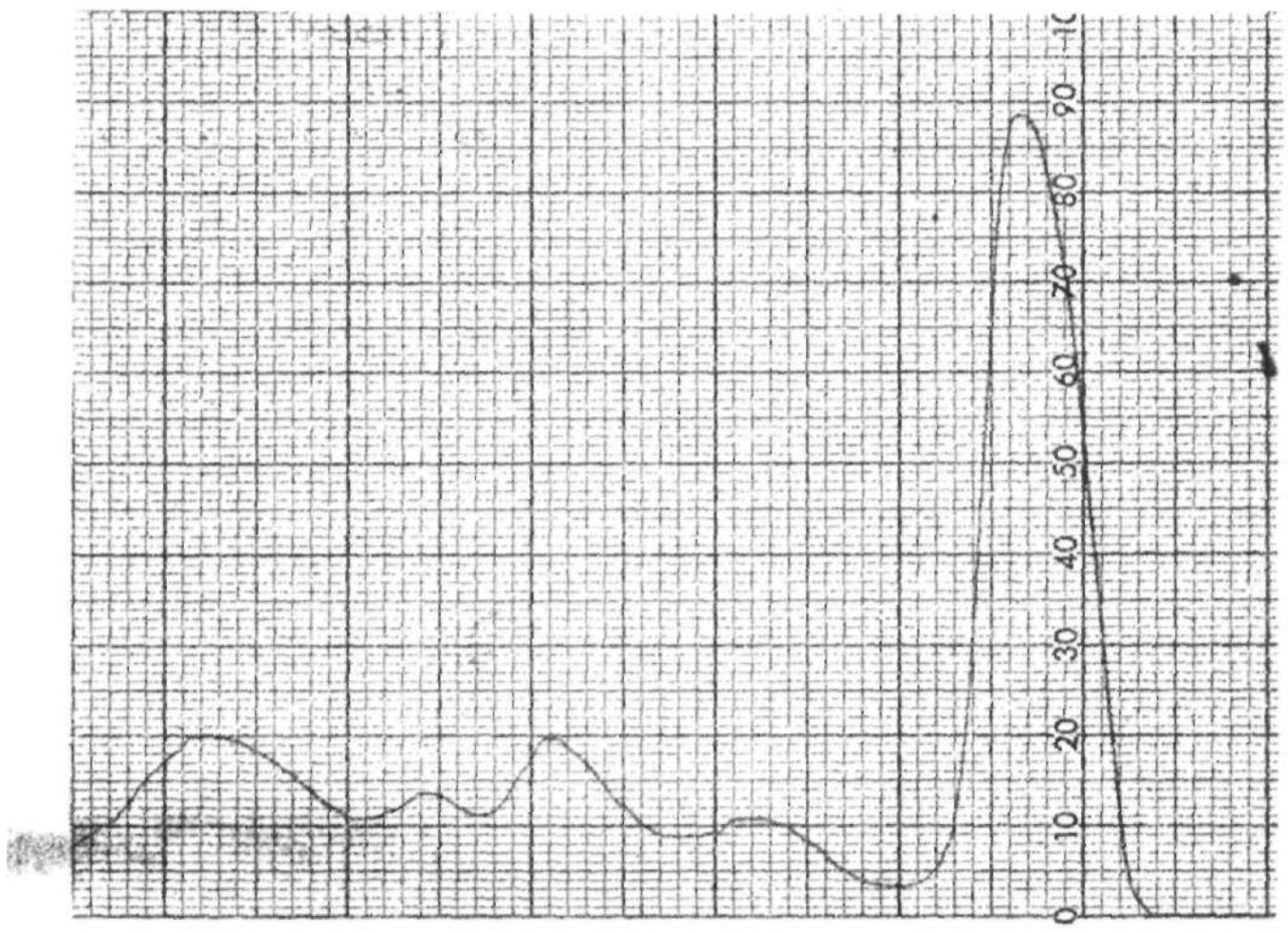

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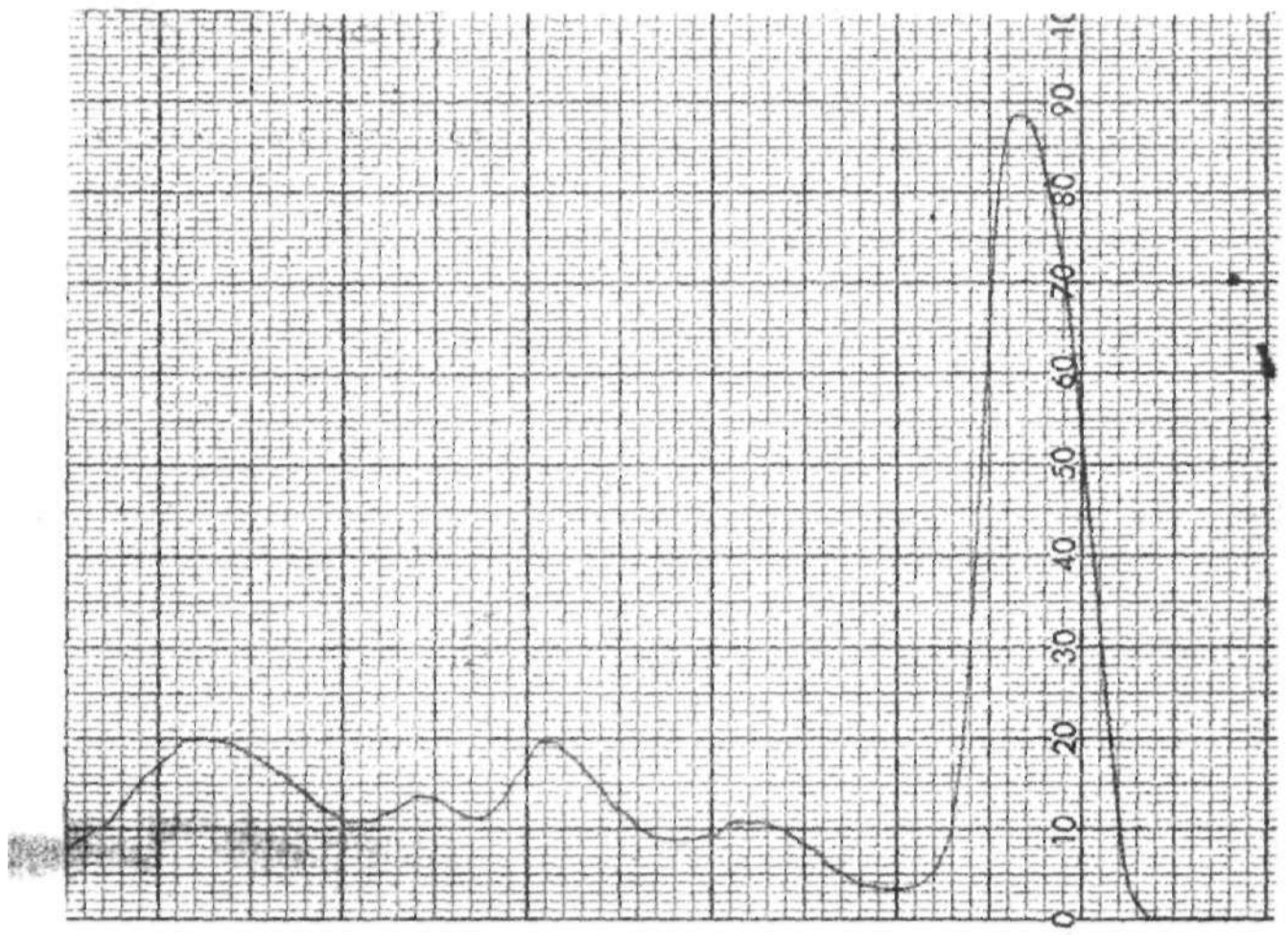

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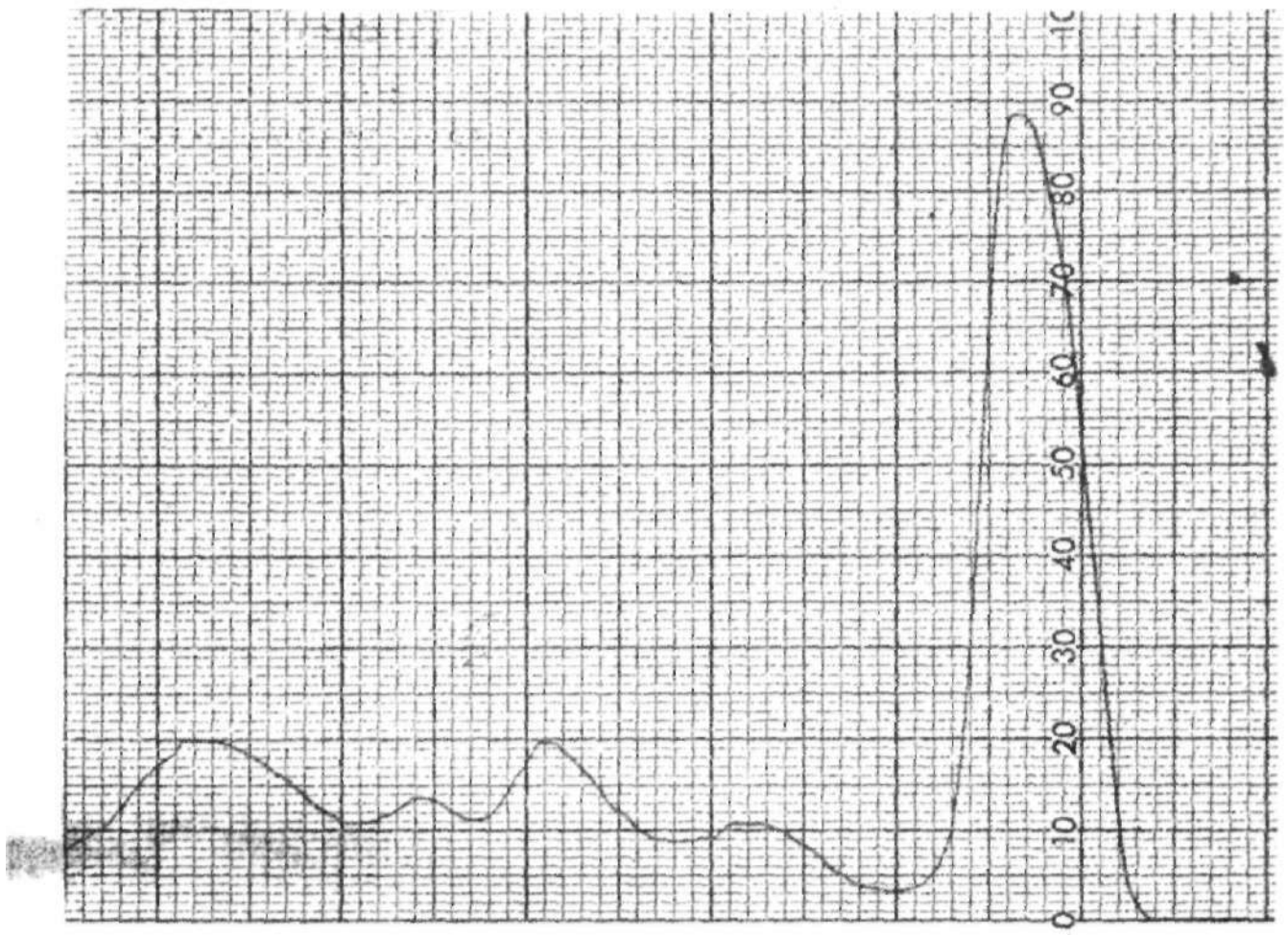

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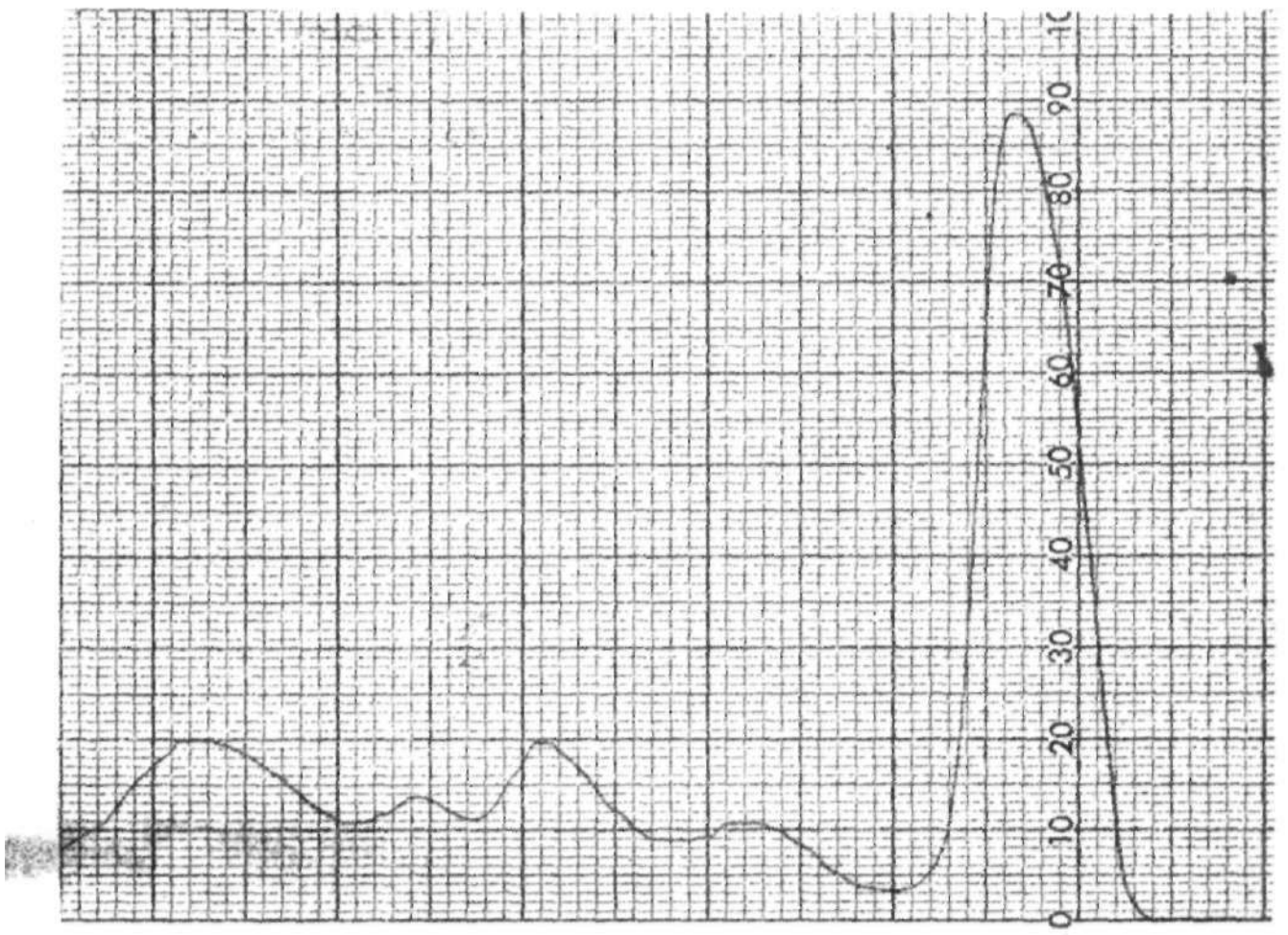

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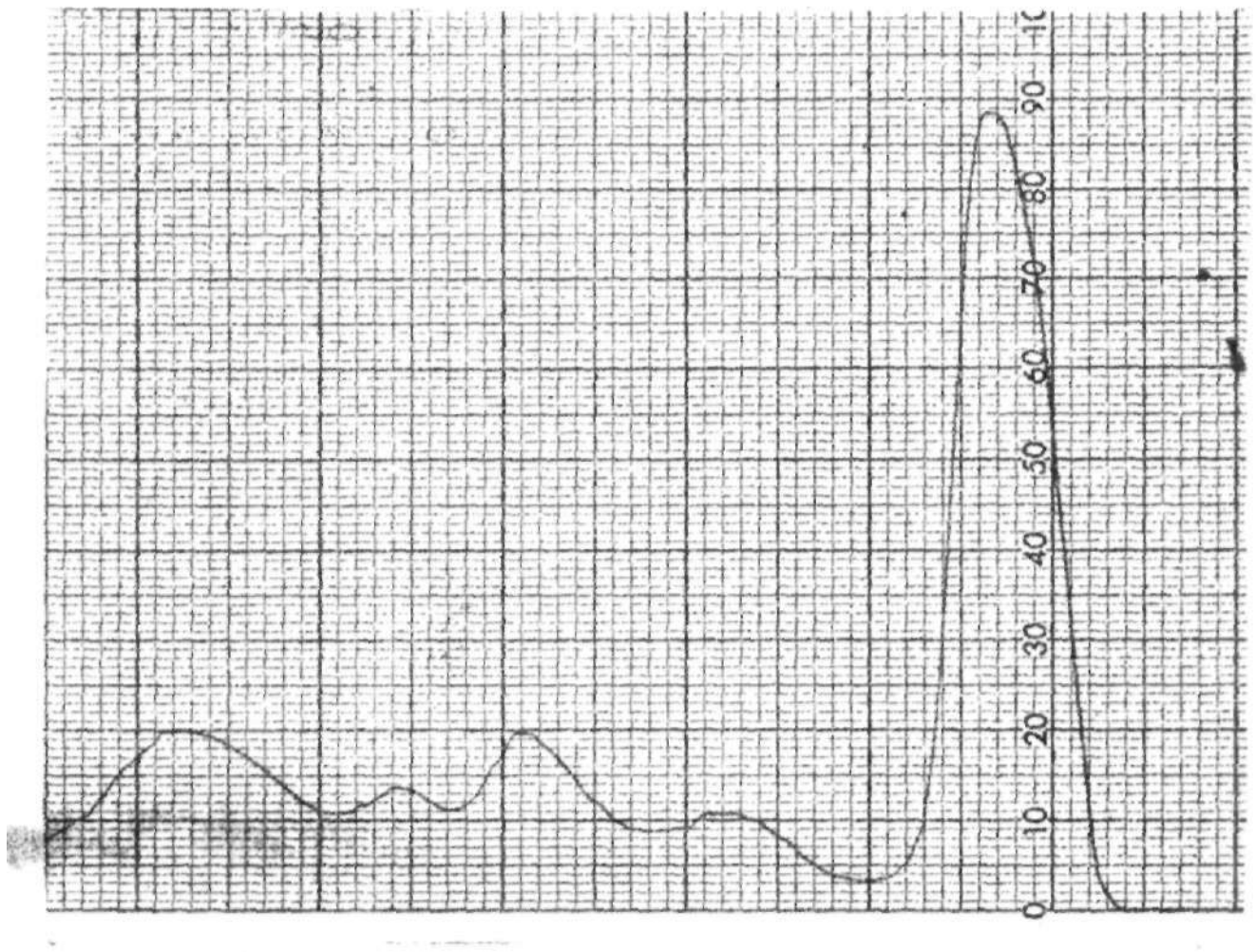

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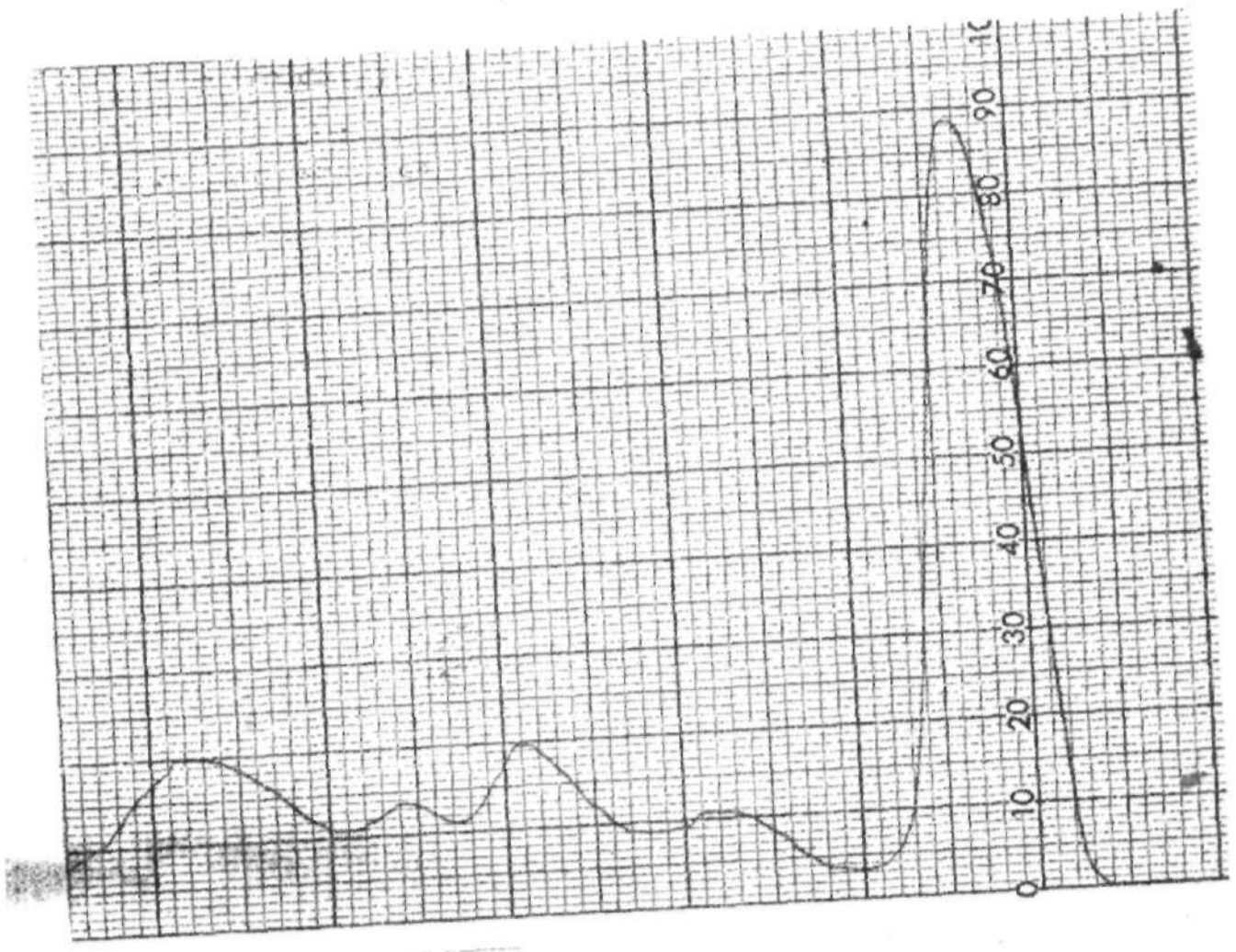

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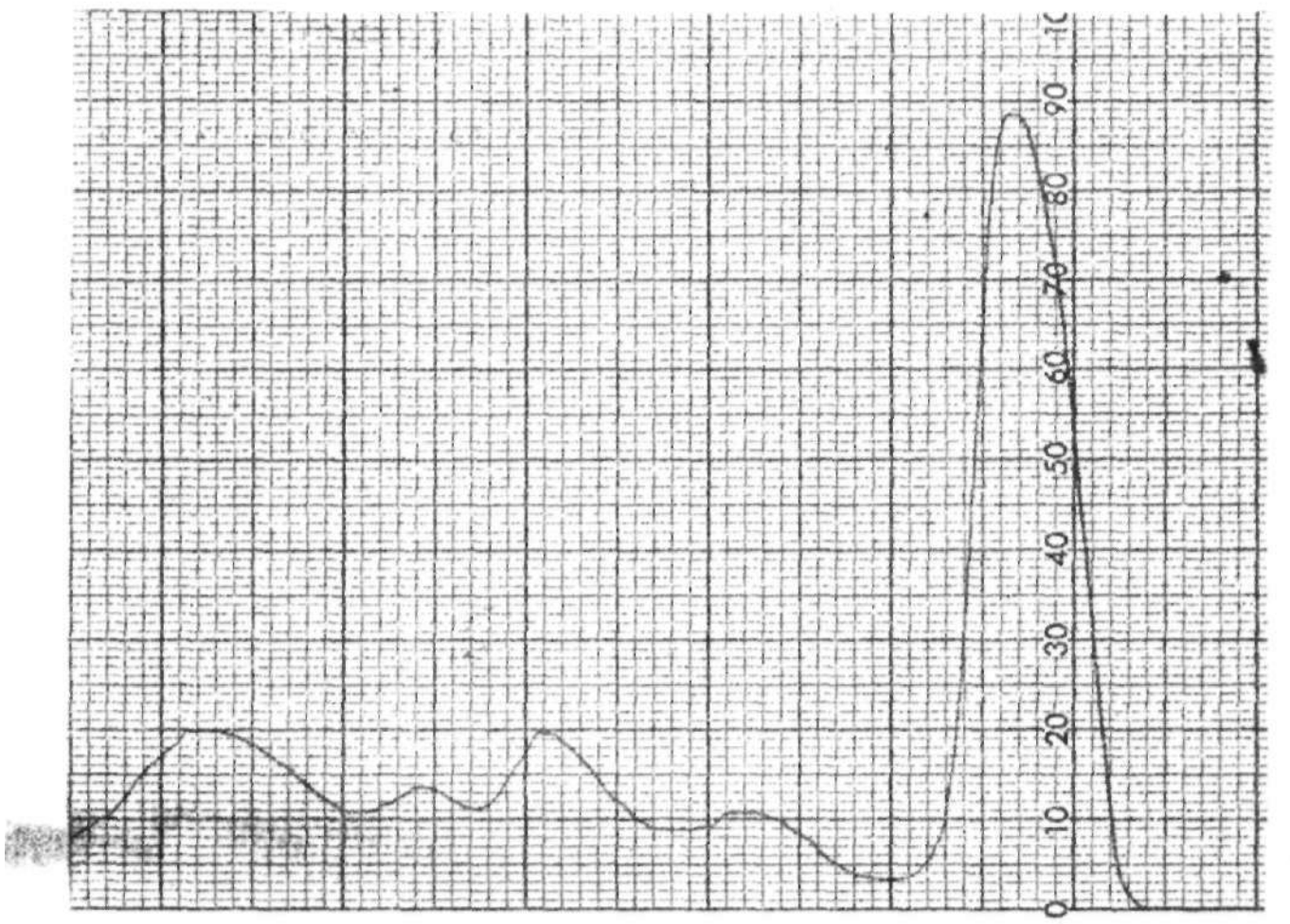

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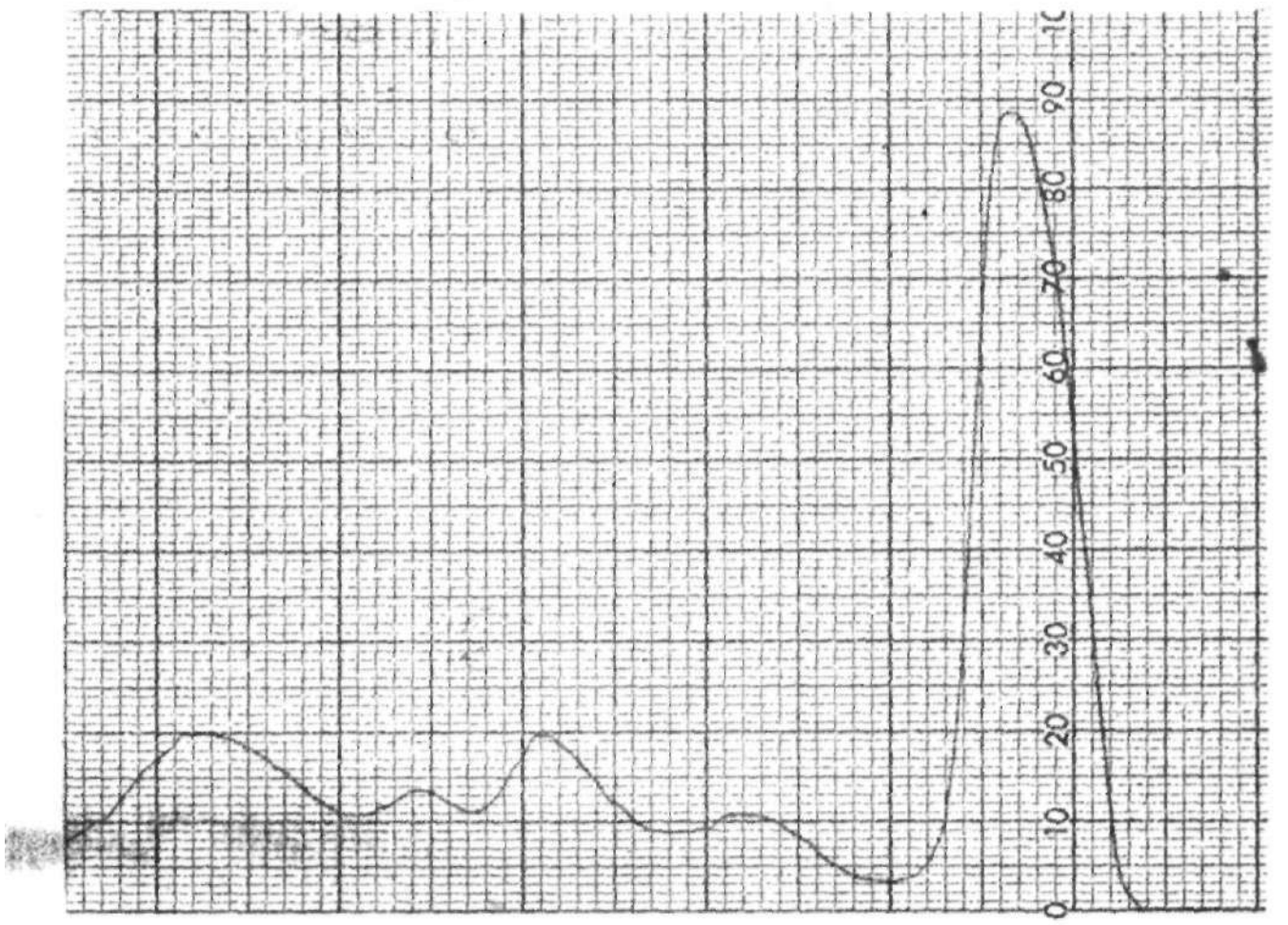

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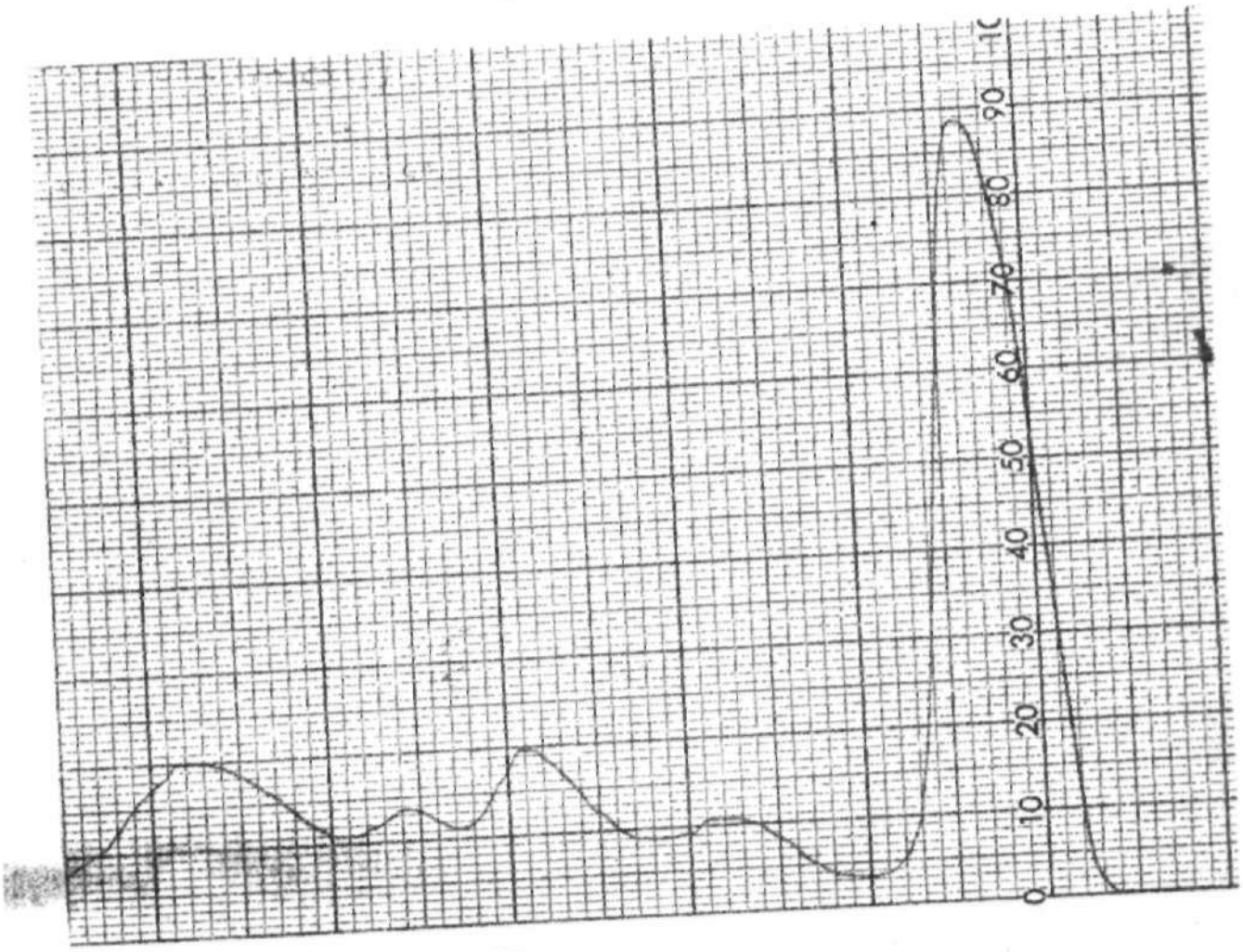
DENSITOMETRIC TRACES OF THE SERUM PROTEIN FRACTIONS.




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