ALTERATIONS IN RAT SERUM PROTEINS IN RELATION TO DIETARY PROTEINS AND B VITAMINS

I. Studies with Growing Rats

BY ANAND G. MULGAONKAR AND A. SREENIVASAN, F.A.SC.
(*Department of Chemical Technology, University of Bombay, Bombay–19*)

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THE relation of dietary proteins to serum proteins in animals has been studied extensively. Differences in the rates of restoration of serum albumin, serum globulin and blood hemoglobin in depleted dogs and rats have been demonstrated as a result of feeding with different types of proteins (Pommerenke *et al.*, 1935; Melnick *et al.*, 1936; Weech and Goettsch, 1938; Madden and Whipple, 1940; Cox and Mueller, 1944; Seeley, 1945; Allison *et al.*, 1946; Robscheit-Robbins and Whipple, 1949), and with synthetic mixtures of amino acids (Robscheit-Robbins *et al.*, 1947; Sebrell and McDaniel, 1952). However, the influence of dietary protein quality on serum globulin fractions is not well defined. Chow *et al.* (1948) observed that enzymatic hydrolyzates of casein and lactalbumin were equally effective in restoring plasma albumin and γ-globulins but that casein was superior to lactalbumin in restoring β-globulins and fibrinogen, in protein depleted dogs. Damodaran (1953) compared the electrophoretic patterns of serum proteins of rats maintained on a 3 per cent. pulse protein diet with those of rats maintained on an 18 per cent. casein diet and observed that the former group had comparatively low concentrations of serum proteins although the relative concentrations of the different fractions were the same as those of animals on the casein diet.

There are indications that vitamins affect the serum protein profile. Greenberg *et al.* (1952) observed that hypoalbuminemia in monkeys in partial or complete deficiencies of vitamin B_6 and nicotinic acid was associated with a rise in γ-globulin level and, in choline deficiency, with elevated level of β-globulin. Similar observations were made by Fisher and Garrity (1953) who reported a decrease in albumin and increase in α- and β-globulins in sera of choline deficient rats.

Considerable data are available on antibody synthesizing ability of vitamin deficient animals (Axelrod and Pruzansky, 1955). Axelrod and coworkers (*see* Axelrod, 1953) have presented a rough classification of the effect of 10 deficiency states in rats upon antibody production according to which severe, moderate and no impairment results from deficiencies, respectively, of pantothenic acid, pyridoxine and folic acid (PGA), riboflavin, thiamine, biotin, vitamin A and niacin-tryptophan, and vitamin B_{12} and vitamin D.

Since B vitamins in general and vitamin B_{12} and PGA in particular, have been implicated, through their roles in the metabolism of amino acids and nucleotides, in general protein metabolism, it was of interest to study the changes in rat serum proteins caused by (i) a general deficiency of B vitamins and, particularly, of vitamin B_{12} and PGA; and (ii) variations of dietary protein quality and level.

Experimental

Two different sets of experiments were carried out. The first one was aimed at ascertaining the influence of certain of the B vitamins on the utilization of amino acids from egg albumin and casein. Weanling male rats (Wistar strain) 40 g. in weight were divided into two groups of twelve each and maintained on purified diets with either devitaminised casein or egg albumin as sole source of protein at 18 per cent. level. Other additions (per cent.) were maize starch 72, arachis oil 3, shark liver oil 2, vitaminised sucrose 1, and salt mixture (U.S.P. XIV) 4. The arachis oil used was fortified with α-tocopherol and vitamin K (Menadione, Merck) such that 100 g. of the diet provided 12·5 mg. and 2·5 mg. respectively of the two vitamins. Each group was subdivided into two, receiving the B vitamins in the vitaminised sucrose at minimal and optimal levels as follows:

	Minimal (mg./kg. diet)	Optimal (mg./kg. diet)
Thiamine hydrochloride ..	0·15	3
Riboflavin	0·15	4
Pyridoxine hydrochloride ..	0·15	3
Calcium pantothenate	1	10
Nicotinic acid	1	20
Inositol	50	200
p-Aminobenzoic acid	50	100
Choline chloride	50	200
Biotin	Nil	0·5
PGA	0·03	1
Vitamin B_{12}	Nil	0·15

It has been ascertained in this as well as in other experiments (Marfatia and Sreenivasan, in press) that throughout the experimental period of 8 weeks, none of the animals in the group receiving the low level of B vitamins shows any gross or clinical symptoms of deficiency of any single B vitamin. Animals were sacrificed after 8 weeks of *ad libitum* feeding.

In the second set of experiments, purified casein and defatted hot-alcohol extracted peanut meal were used as protein sources at 10 and 18 per cent. levels and the effects of PGA and vitamin B_{12} were studied. Young male rats, 50 g. in weight, were first depleted of their vitamin B_{12} and PGA reserves by feeding an iodocasein diet consisting of (in g./100 g. of the diet): vitamin-free casein 10, iodinated casein ('Protomone', Cerophyl Laboratories, Kansas City, Mo.) 0·15, arachis oil 6, shark liver oil 2, sucrose 9·85, maize starch 68, and salt mixture (U.S.P. XIV) 4. The sucrose provided the following vitamin levels (in mg./kg. of the diet): thiamine hydrochloride 6, calcium pantothenate 20, pyridoxine hydrochloride 6,r iboflavin 10, biotin 1, nicotinic acid 30, choline chloride 500, inositol 500, α-tocopherol 50, and vitamin K 10. These were considered adequate for the hyperthyroid condition.

At the end of 4 weeks, the animals were divided into groups of 6. One group was continued on the same diet modified by the omission of the iodinated casein and inclusion of 2 per cent. succinyl sulphathiazole. A second group was fed the casein at 18 per cent. level with adjustment against the maize starch. Two similar groups were with peanut meal at 10 and 18 per cent. protein levels. There were corresponding groups in each case receiving supplements of vitamin B_{12} and PGA at levels of 50 μg. and 5 mg. respectively per kg. of the diet. Besides these 8 groups, there were four additional ones with the casein or peanut protein diets, at 10 per cent. protein level, and with single additions of vitamin B_{12} or PGA at levels same as above. Growth was recorded over a five-week period of *ad libitum* feeding when the animals were sacrificed.

At the conclusion of the respective experimental periods the animals were anesthetised and, blood drawn from the inferior vena cava using a dry syringe was allowed to clot at 37° for one hour in sterile glass container. The separated serum was obtained by centrifugation at 2°. Livers were quickly excised and chilled in cracked ice.

Total serum protein was determined by the biuret method (Gornall *et al.*, 1949).

Paper electrophoresis.—Fractionation of serum proteins was carried out by electrophoresis on Whatman No. 3 paper strips, 5×34 cm., in a horizontal open-strip type cell with barbiturate buffer of pH 8·6 and ionic strength 0·075. Ten μl. serum was used and separation was effected at a constant current of 2·5 mA./strip for 16 hours at room temperature (28° C.). The subsequent procedure was as described by Jencks *et al.* (1955). The strips were dried in an air oven at 110° C. for 30 minutes and later stained for 16 hours in an aqueous bath containing 0·01 per cent. bromophenol blue, 5 per cent. glacial acetic acid and 5 per cent. zinc sulphate. Excess dye was removed by washing the strips in 2 per cent. glacial acetic acid solution, renewed at intervals of 5 minutes, for 20 minutes. A final rinse for 2 minutes in a solution containing 2 per cent. sodium acetate and 10 per cent. glacial acetic acid was followed by blotting and drying at 110° C. The strips were scanned after immersion in hot liquid paraffin, draining for 2 hours and blotting off excess paraffin. An 'EEL' scanner (Evans Electroselenium Ltd., Harlow, Essex) was used for the purpose.

Liver vitamin B_{12} was determined after liberation from the tissue by overnight incubation under toluene with papain (B.D.H., 25 mg./g. of liver) in 0·1 M acetate buffer of pH 4·6; the samples were then autoclaved, homogenized, neutralized and made to volume. Assays were carried out with *Lactobacillus leichmannii* (ATCC 7830) by a turbidimetric adaptation of the U.S.P. method (Rege, 1953).

Liver PGA was determined after autolysis in 0·2 M phosphate buffer of pH 7·6 using *Streptococcus fæcalis* R. (ATCC 8043) and the assay medium of Mitbander and Sreenivasan (1954).

Results

Food Intake and Growth

Although no pair-fed control animals were kept, the excess food intakes of the various controls over the corresponding experimentals were only 5–8 per cent. on high protein (18 per cent.) diets and from 15–20 per cent. on low protein (10 per cent.) diets. On the latter diets the consumption was 5–6 per cent. higher in the peanut protein group than in the corresponding casein group whereas no significant differences were observed between the intakes of egg albumin and casein and between those of casein and peanut protein when fed at 18 per cent. level. Food intakes at higher level of proteins were from 7–8 per cent. higher than at lower level.

The results presented below are as may be modified by these differences in food intakes. The differences in the intake of proteins being even smaller

TABLE I

Effect of variations in dietary protein quality and B vitamins on growth

In experiment 1, rats were reared for 8 weeks on purified diets of egg albumin or casein at 18 per cent. level with supplements of B vitamins either at optimal or at minimal levels. In experiment 2, rats deprived of their Vitamin B_{12} and PGA reserves were reared for 5 weeks on vitamin B_{12} and PGA free casein or peanut protein diets, at 10 or 18 per cent. protein level, with or without supplements of vitamin B_{12} and/or PGA.

The results are expressed as mean weight gains (g.) per week ± standard deviation.

Protein quality	Protein level %	Optimal/control	Minimal/deficient
Experiment 1:		B vitamins	
Egg albumin ..	18	18·2±3·2	15·4±2·0
Casein ..	18	20·0±2·6	12·0±1·8
Experiment 2:		Vitamin B_{12} and PGA	
Casein ..	18	42·6±3·8	36·7±4·6
Peanut protein ..	18	34·8±4·8	27·6±4·2
Casein ..	10	12·2±4·7	4·6±2·2
Peanut protein ..	10	14·0±1·9	3·2±2·8
		Vitamin B_{12}	
Casein ..	10		8·8±1·3
Peanut protein ..	10		9·1±2·4
		PGA	
Casein ..	10		6·4±3·1
Peanut protein ..	10		8·2±1·0

may not therefore be expected to modify the interpretation of the results reported.

The growth data for the two experimental series are summarized in Table I. Table II includes the liver levels of PGA and vitamin B_{12} in deficient and control animals of the second experimental series. It is seen that the improvement in growth resulting from optimal intake of B vitamins in experiment 1 is more marked in the casein than in the egg albumin group.

TABLE II

Vitamin B_{12} and PGA deficiencies

Liver levels of the vitamins in rats on deficient diets of casein or peanut protein at 10 or 18 per cent. protein level, with or without supplements of vitamin B_{12} and/or PGA.

Results are mean values for 6 rats ± standard deviation.

Group	Protein level %	Casein: Liver PGA μg./g.	Casein: Liver vitamin B_{12} mμg./g.	Peanut protein: Liver PGA μg./g.	Peanut protein: Liver vitamin B_{12} mμg./g.
			Vitamin B_{12} and PGA		
Control ..	18	8·2±0·5	121·3±19·2	7·4±0·6	80·7±13·4
Deficient ..	18	3·0±0·7	35·9±21·4	2·1±0·9	14·5± 7·9
Control ..	10	6·8±0·6	92·6± 8·7	4·3±0·2	58·7± 9·8
Deficient ..	10	2·1±0·3	26·8±11·4	1·4±0·5	17·3± 4·2
			Vitamin B_{12}		
Deficient ..	10	5·4±0·5	29·7± 9·1	3·9±0·4	15·3± 4·2
			PGA		
Deficient ..	10	2·5±0·8	84·8± 7·2	1·8±1·1	60·3± 6·6

While growth retardation (Table I) and other deficiency manifestations (Table II) in single and combined deficiencies of vitamin B_{12} and PGA are as may be expected, the effects due to feeding of these vitamins are again more marked in the peanut protein than in the casein groups, particularly at lower intake of proteins. It is interesting to note that liver levels of the vitamins are higher in the casein-fed than in the corresponding peanut protein-fed groups.

Serum Proteins

The fractions labelled " Albumin " " α_1- ", " α_2- ", " β- " and " γ- " " globulins " are defined by the electrophoretic pattern illustrated in Fig. 1. In all cases, the significance of differences was calculated from data using individual pairs of results and evaluating " t ". The corresponding " p " values were read from standard tables (Fisher and Yates, 1948) at the appropriate degrees of freedom. The differences were considered significant for values of " p " less than 0·05, and highly significant for values less than 0·01.

(*a*) *Relation to dietary proteins.*—Table III demonstrates the effects due to variations in dietary protein quality and level on serum proteins. Protein quality chiefly influences serum levels of albumin and α_1-globulin. Thus with egg albumin, the serum albumin level is higher than with casein

TABLE III

Influence of dietary protein quality and level on rat serum proteins

Figures represent the serum protein levels of rats fed on diets with optimal levels of B vitamins (experiment 1) and with PGA and vitamin B_{12} supplemens (experiment 2). For details of the experiments refer Table I. Results are mean values for 6 rats with standard deviations.

Group	Protein level in diet %	Total protein	Albumin	Globulins α_1	α_2	β (g./100 ml. serum)	γ
Experiment 1:							
Egg albumin ..	18	6·14 ±0·16	2·42 ±0·06	1·06 ±0·03	0·57 ±0·04	0·99 ±0·04	1·09 ±0·09
Casein ..	18	6·24 ±0·07	2·25 ±0·05	1·29 ±0·07	0·65 ±0·03	1·03 ±0·07	1·01 ±0·09
Experiment 2:							
Casein ..	18	7·00 ±0·20	2·58 ±0·18	1·52 ±0·29	0·74 ±0·09	1·11 ±0·09	1·0[illegible] ±0·0[illegible]
Casein ..	10	5·35 ±0·31	2·13 ±0·05	0·91 ±0·07	0·36 ±0·07	0·97 ±0·08	0·97 ±0·11
Peanut protein ..	18	5·90 ±0·47	2·30 ±0·23	1·15 ±0·05	0·66 ±0·08	0·95 ±0·04	0·84 ±0·07
Peanut protein ..	10	5·35 ±0·26	2·00 ±0·12	0·80 ±0·03	0·36 ±0·04	1·03 ±0·13	1·08 ±0·04

($p < 0{\cdot}05$), and peanut protein is inferior to casein in this respect ($p < 0{\cdot}05$). On the other hand, α_1-globulin concentration is higher in the casein-fed than either in the egg albumin-($p < 0{\cdot}001$) or peanut protein-fed ($p < 0{\cdot}001$) groups. The α_2-globulin level is also higher ($p < 0{\cdot}05$) in the casein as compared to the egg albumin group whereas its levels in the casein and peanut protein groups are not appreciably different. β- and γ-globulin levels are higher ($p < 0{\cdot}05$) with casein than with peanut protein, but only at 18 per cent. level, no significant differences existing in this respect at the lower level and between egg albumin and casein groups.

Except for an observed difference ($p < 0 \cdot 001$) between the 18 per cent. casein and peanut protein groups, protein quality, apparently, does not influence total serum protein concentration. On the other hand, there is lowering in total serum protein in low-level protein diets. This results from significant drops in levels of albumin, α_1-, and α_2-globulins, in the casein as well as in the peanut protein groups.

Variations in dietary protein quality, however, does not alter the relative distribution of serum protein fractions and although with low-level protein diets there is a relative lowering of α-globulins with rise in β- and γ-globulins, the albumin-globulin ratio remains unaltered.

(*b*) *Effects of B vitamins.*—A low intake of B vitamins causes a drop in total serum proteins, the extent of which is, apparently, influenced by dietary protein quality (Table IV). The depression is marked in the casein-fed rats ($p < 0 \cdot 01$), while those receiving egg albumin show a small but insignificant change. The reduction is largely confined to the γ-globulin fraction and is highly significant ($p < 0 \cdot 001$) for either group. In casein-fed rats there is, additionally, a marked drop ($p < 0 \cdot 001$) in α_1-globulin level.

(*c*) *Alterations in single and combined deficiencies of vitamin B_{12} and PGA* (*Table IV*).—There is a drop ($p < 0 \cdot 05$) in serum protein level in combined deficiency of vitamin B_{12} and PGA, especially on low protein diets ($p < 0 \cdot 01$). In the casein-fed groups, deficiency causes reductions in serum levels of albumin ($p < 0 \cdot 01$) and γ-globulin ($p < 0 \cdot 001$) at high protein intake. With lower intake, there are greater reductions ($p < 0 \cdot 001$) in serum levels of albumin, α_1-globulin and γ-globulin. In the deficient animals receiving peanut protein there is a marked reduction in γ-globulin level ($p < 0 \cdot 01$), especially with low intake of protein ($p < 0 \cdot 001$). This reduction is associated with appreciable lowering of albumin ($p < 0 \cdot 05$) and β-globulin ($p < 0 \cdot 01$) levels on low protein diet, and of α_1-globulin ($p < 0 \cdot 005$) level on high protein diet.

In vitamin B_{12} deficiency alone, there are marked reductions in albumin ($p < 0 \cdot 001$) and α_1-globulin ($p < 0 \cdot 01$) levels in the casein-fed rats, whereas, in the peanut protein-fed group α_2-globulin level is singly affected ($p < 0 \cdot 01$).

In single PGA deficiency, with casein in the diet, there is a decrease in albumin level ($p < 0 \cdot 01$) along with marked reductions ($p < 0 \cdot 001$) in β- and γ-globulin levels. In the peanut protein-fed rats, on the other hand, only the γ-globulin fraction is affected ($p < 0 \cdot 01$).

TABLE IV

Influence of B vitamins on rat serum protein fractions

Data indicate the deviations in the levels of serum protein fractions caused by (i) a minimal intake of all B vitamins (experiment 1), and (ii) single and combined deficiencies of vitamin B_{12} and PGA (experiment 2), under conditions where either the dietary protein quality or level or both are varied. For details of the experiments refer Table I.

Results are mean values for 6 animals ± standard deviation.

Group	Level of protein in diet %	Total protein	Albumin	Globulins α_1	Globulins α_2	Globulins β	Globulins γ
				[Values as percentage of normal (control) groups]			
Experiment 1:				Minimal supplements of B vitamins			
Egg albumin ..	18	92·4± 7·8	91·4±5·6	106·6±7·8	108·7±7·8	93·0± 6·6	78·9±4·3
Casein ..	18	83·3±10·8	95·6±3·8	77·6±5·4	97·6±5·2	93·2±10·3	58·1±9·8
Experiment 2:				Vitamin B_{12} and PGA deficiency			
Casein ..	18	93·6± 3·1	86·9±7·2	96·0±5·0	101·3±3·3	110·9± 4·2	77·9±4·4
Peanut protein	18	91·6± 3·4	99·2±7·1	79·9±6·3	105·1±7·1	94·8± 5·1	81·4±6·8
Casein ..	10	77·8± 6·9	64·8±7·8	58·2±4·4	102·8±6·0	115·4± 6·1	78·4±8·3
Peanut protein	10	83·2± 4·6	89·6±6·4	98·8±7·2	97·3±4·3	86·3± 3·2	62·4±8·0
				Vitamin B_{12} deficiency			
Casein ..	10	86·7± 4·8	71·8±8·3	83·8±6·9	106·5±4·2	101·2± 9·3	105·1±3·3
Peanut protein	10	96·1± 5·4	93·0±3·3	104·1±9·8	84·2±6·1	104·2± 5·6	97·4±7·1
				PGA deficiency			
Casein ..	10	83·2± 4·3	83·9±4·3	93·3±6·9	102·2±9·3	73·2±10·2	70·1±5·9
Peanut protein	10	95·2± 6·9	95·9±7·8	96·9±5·4	91·3±5·6	103·2± 6·7	85·5±4·1

Discussion

The observation that the response to growth with optimal levels of B vitamins is greater in the casein-fed than in the egg albumin group is also borne out from other studies (Marfatia and Sreenivasan, in press) relating to the utilization of dietary amino acids as influenced by B vitamins in general. The depression in serum protein level due to low intake of B vitamins is also greater in the casein than in the egg albumin group. This would suggest that with a superior protein higher levels of B vitamins do not exert an effect comparable to that with a low quality protein.

It is evident that dietary protein quality and level have profound influence on the serum proteins of the rat under normal and in stress conditions. Also, the effects due to these factors are interdependent, the influence of dietary protein quality on serum proteins being modified by protein level in the diet and *vice versa*.

The high values for serum albumin in rats fed egg albumin may be due to a similarity in the amino acid patterns in egg albumin and in serum albumin. The amino acid make-up of casein apparently favours synthesis of α-globulins. On the other hand, the imperfections in amino acid composition of peanut protein reflect upon serum levels of all the fractions, especially on those of albumin and α_1-globulin.

The observation that dietary protein qualitatively and quantitatively influences the serum levels of albumin and α_1-globulin would suggest a greater dependence of these fractions than of other fractions on dietary amino acids.

The constancy of the relative distribution of serum protein fractions in the face of variations in dietary protein quality is in conformity with the findings of Damodaran (1953), although it is seen that dietary protein level causes alterations in globulin fractions, the albumin-globulin ratio remaining unaltered.

The decrease in γ-globulin level with minimal intakes of B vitamins is similar to the impairment in antibody synthesizing ability of the rat known to result from specific deficiencies of certain of the B vitamins, notably, pyridoxine, pantothenic acid and folic acid (Axelrod and Pruzansky, 1955). Similarly, the observation that γ-globulin decreases in PGA deficiency but not in vitamin B_{12} deficiency is comparable with the impairment in antibody synthesizing ability of the rat in PGA deficiency and the absence of such impairment in vitamin B_{12} deficiency (Ludovici and Axelrod, 1951).

SUMMARY

The alterations in rat serum proteins resulting from (i) variations in dietary protein quality and level, (ii) minimal intakes of B vitamins and (iii) single and combined deficiencies of vitamin B_{12} and folic acid have been studied with egg albumin, casein and peanut protein diets at 10 and 18 per cent. protein levels.

Protein quality chiefly influences serum concentrations of albumin and α_1-globulin, the effects on other fractions being variable. Protein level affects serum levels of albumin, α_1-globulin and α_2-globulin. The effects due to these dietary variables are interdependent.

Variations in dietary protein quality do not alter the relative distribution of serum protein fractions. Changes in protein level cause shifts in the relative distribution of globulins, the albumin-globulin ratio remaining unaltered.

The nature and extent of changes in serum protein profile due to vitamin insufficiencies or deficiencies are also influenced, qualitatively and quantitatively, by dietary amino acids. With minimal intakes of B vitamins γ-globulin is chiefly reduced, but other fractions, particularly α_1-globulin, may also be affected. In a combined deficiency of vitamin B_{12} and folic acid a common reduction in γ-globulin is variably attended by reductions in albumin and in α_1- and β-globulins. In single vitamin B_{12} deficiency, the changes are confined to albumin, α_1-globulin and α_2-globulin fractions, whereas single folic acid deficiency chiefly affects γ-globulin with variable effects on albumin and β-globulin fractions.

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A. Sreenivasan

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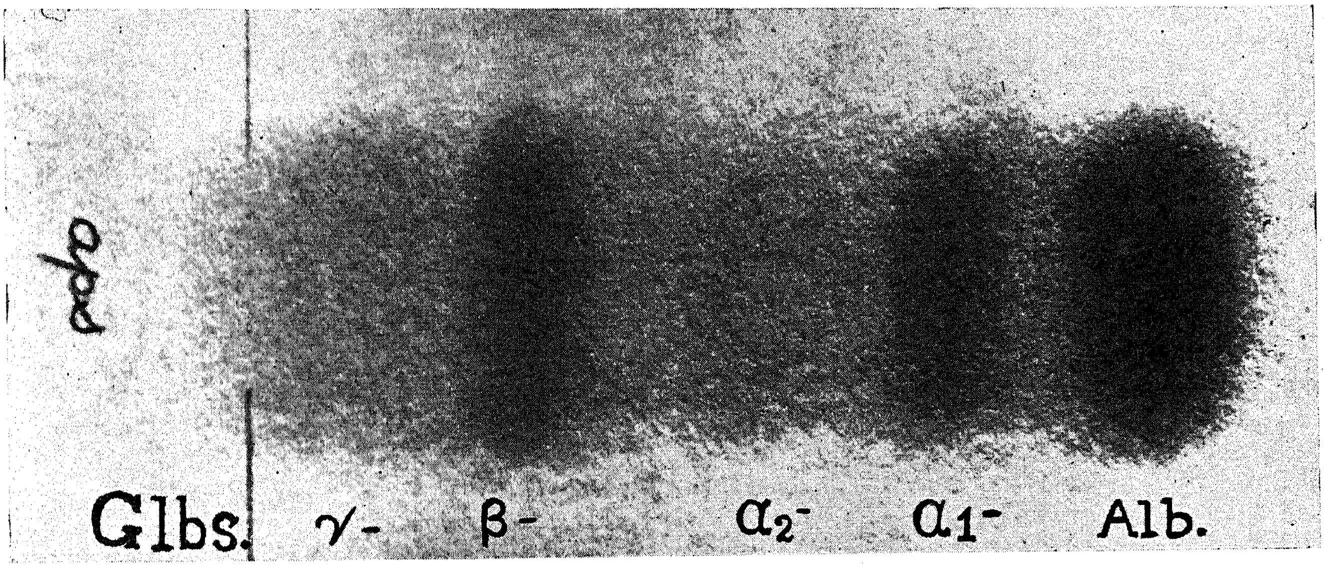

FIG. 1. A typical electrophoretic profile of rat serum protein fractions.

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