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INFLUENCE OF VITAMIN B_6 DEFICIENCY ON SERUM PROTEINS IN LAYING CHICKENS

By
MOHAMMAD WAFIK ATTAR

A THESIS

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INFLUENCE OF VITAMIN B_6 DEFICIENCY ON
SERUM PROTEINS IN LAYING CHICKENS

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VITAMIN B_6 AND SERUM PROTEINS

ATTAR

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AN ABSTRACT OF THE THESIS OF

Mohammad Wafik Attar for M.S. in Poultry Nutrition

Title: Influence of vitamin B_6 deficiency on serum proteins in laying chickens.

Pyridoxal phosphate, the active form of vitamin B_6, is now recognized as the coenzyme for a great variety of enzymes involved in catalyzing various metabolic transformations. The most important of these are amino acid transformations. Previous work has also indicated that vitamin B_6 deficiency impairs antibody formation.

The present study was conducted to investigate the influence of vitamin B_6 deficiency on serum proteins in laying chickens. Development of B_6 deficiency in mature White Leghorn females resulted in loss of body weight, reduced feed intake, and complete cessation of egg production.

Serum glutamic oxalacetic transaminase (SGO-T) activity in pyridoxine deficient birds was considerably lower than that of birds fed B_6-adequate diets. No statistically significant differences in serum total nitrogen were observed between B_6-deficient and control birds. Serum non-protein-nitrogen was increased as a result of the B_6 deficiency. No significant differences were found in albumin globulin ratios of experimental and control birds.

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I. INTRODUCTION

During the past few decades, there has been growing emphasis on basic research in the agricultural sciences. In the field of nutrition, the problem of nutrient interrelationships has received the attention of many nutritionists. Among these nutrients, the vitamins stand out as an important class with a basic role in general metabolism.

Pyridoxine, the water-soluble vitamin , is considered the building unit for the coenzyme, pyridoxal phosphate. Pyridoxal phosphate is now established as the coenzyme for many enzymes involved in catalyzing amino acid transformations such as transamination, decarboxylation and racemization.

Many nutritional studies concerning the relationship of pyridoxine to amino acids and protein metabolism have been carried out with various experimental animals. The chicken has been frequently used as an experimental animal, because of its well known nutritional requirements.

Studies on vitamin B_6 deficiency in the laying chicken and its effect on total serum proteins and serum glutamic-oxalacetic transaminase (SGO-T) activity have not been reported in the literature. Few studies have been

conducted however, regarding the vitamin B_6 requirement of laying chickens. These studies have demonstrated the importance of vitamin B_6 in the nutrition of the laying hens, and have shown that severe pyridoxine[1] deficiency results in complete cessation of egg production.

The main purpose of the present study was to investigate the effect of vitamin B_6 deficiency on serum proteins and SGO-T activity in the laying chicken. Total serum protein was determined by analyzing the serum for total nitrogen and non-protein-nitrogen. The paper electrophoresis technique was used to study the protein fractions of the serum.

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1. Pyridoxine and vitamin B_6 have been used interchangeably throughout the thesis.

II. REVIEW OF LITERATURE

Glutamic Oxalacetic Transaminase and Vitamin B_6

Numerous reports have been published concerning the effect of vitamin B_6 deficiency on glutamic oxalacetic transaminase (GOT) activity in mammalian blood. There is relatively little information, however, about the effect of B_6 deficiency on GOT in the blood of birds.

Babcock (1959) observed a considerable reduction in the SGO-T activity in vitamin B_6 deficient rats. He showed that B_6 was incorporated into GOT within 24 hours after it was fed.

Talageri *et al*. (1961) reported that there was a moderate reduction in the level of blood amd liver GOT in rats fed diets deficient in vitamin B_6. When desoxy-pyridoxine, a pyridoxine antimetabolite, was added to the B_6 deficient diet there was a marked decrease in the levels of GOT as compared to pair fed control. They also observed that when the B_6 deficient rats were placed on the B_6 adequate diet for 5 weeks, the GOT values were comparable to those of the control.

Tulpule *et al*. (1959) investigated the effects of low protein, and adequate protein with or without pyridoxine supplementation on blood GOT activity in young rats. They

observed reduction in GOT values in blood of rats fed diets low in protein or deficient in pyridoxine. This effect was more marked in the combined deficiency of these 2 nutrients. However, the GOT activity returned to normal in 2-3 weeks when the missing nutrients were added to the diet.

The results of studies conducted by Brin et al. (1960) showed the same depressing effect of vitamin B_6 deficiency on plasma GOT in growing and adult rats. They reported that the depression of plasma GOT level was 63% below the normal value in the case of young rats, while it was 22% in the adult. The SGO-T activity in deficient rats was rapidly restored to normal by injecting pyridoxal HCl, or by feeding the deficient rats a pyridoxine adequate diet.

Yeh _et al_. (1960) conducted studies on the plasma GOT levels in young male rats with chronic and intermittent B_6 deficiencies. They reported that plasma GOT decreased steadily in the rats with chronic B_6 deficiency, while plasma GOT level of the adequate group was within a normal range. Their results showed a very close relationship of plasma GOT levels and B_6 supplementation. Moreover, they observed that rats of the intermittent B_6 deficiency group showed decreased or increased values of plasma GOT in accordance with depletion or repletion of vitamin B_6.

In experiments carried out by Kuchinskas _et al_. (1957) the vitamin B_6 deficiency state evoked in rats by

administering L-Penicillamine, with anti-B_6 activity, caused a reduction in GOT activity in the livers. This inhibitory effect was reversed by administering in vivo pyridoxine, or by adding in vitro pyridoxal-5-phosphate. Cadwell et al. (1953) studied the GOT activity in livers of rats fed different amounts of casein in the diet, in the presence and absence of dietary pyridoxine. They observed a reduction in GOT activity as a result of B_6 deprivation. The GOT activity varied also with the protein content of the diet, and was elevated when feed intake was restricted provided that dietary B_6 was supplied.

Rosen et al. (1960) reported that the activity of GOT was reduced to 80% of the normal in the brain and liver of rats fed a vitamin B_6 deficient diet. The GOT level was restored to its normal value after injecting the deficient rats with B_6.

Recent studies indicate that the effect of pyridoxine deficiency on GOT level in human plasma is not very great. Plough (1960) reported a slight, but non significant decrease in SGO-T activity in human subjects fed a vitamin B_6 deficient diet for 3 weeks. Babcock et al. (1960) observed a wide range of SGO-T activity values in human subjects fed a diet low in pyridoxine. The SGO-T values observed fell within the normal range of variation. Because of this they concluded that single SGO-T determination is not sufficient to evaluate the degree of B_6

deficiency. They found that the increase in SGO-T values on repletion with 5 to 10 mg of vitamin B_6 per day was more indicative of pyridoxine deficiency. Raica and Sauberlich (1964) similarly concluded that plasma GOT activity in man cannot be used to establish the presence of a vitamin B_6 deficiency.

Few studies have been reported concerning the effect of pyridoxine deficiency on the activity of GOT in chickens.

Goswami and Robblee (1958) carried out studies on the GOT activity in tissues of chicks fed diets of varying B_6 content. There was a considerable reduction in GOT activity in blood, liver, and heart of chicks fed a pyridoxine deficient diet.

Daghir and Balloun (1963) reported that chicks receiving a vitamin B_6 deficient diet had about 45% of the SGO-T activity of those receiving diets adequate in B_6. They observed a significant increase in SGO-T activity as the level of B_6 in the diet is increased up to an optimal level beyond which no more significant increase in SGO-T results. In their studies, they demonstrated that the SGO-T activity varies with the breed of chickens, especially when the vitamin B_6 content of the diet is marginal.

Serum Proteins and Vitamin B_6

The role of the coenzyme form of vitamin B_6 in amino acid metabolism is well established. Substantial evidence on the relation of B_6 to amino acid metabolism has been obtained in many studies which have been adequately reviewed by Snell (1958).

No information has been found in the literature regarding the effect of vitamin B_6 deficiency on serum proteins in chickens. Few studies conducted with mammalian species, however have been reported.

In a study by Beaton *et al*. (1953), the levels of total serum protein in pyridoxine deficient and adequate rats were investigated. These workers induced pyridoxine deficiency in rats by feeding them a pyridoxine-free diet together with desoxypyridoxine for 21 days. The control rats were pair-fed a pyridoxine adequate diet in order to eliminate the effects of inanition. These workers observed no differences in the concentrations of total serum proteins between the vitamin B_6 deficient and the control rats. They noted a slight non-significant change of albumin/globulin ratio due to B_6 deficiency. These findings were confirmed later by Myrtle and Pike (1960) also with rats.

Vilter *et al*. (1953) investigated the effect of vitamin B_6 deficiency induced by deoxypyridoxine on serum

proteins in adult human patients. They found no alterations in total serum proteins as a result of pyridoxine deficiency.

The results of these studies indicate no influence of the pyridoxine deficiency state on the level of total serum proteins in some mammalian species.

Numerous investigators were more interested in the changes of individual fractions of serum protein rather than in the changes of total serum proteins under pyridoxine deficiency conditions. Extensive studies have been conducted on the relation of vitamin B_6 and antibodies, which are a specific kind of serum proteins. The nutritional requirement of pyridoxine for antibody formation was first established by Stoerk and Eisen (1946). They observed that immunized rats fed a pyridoxine deficient diet developed antibody levels in the serum far below those of either inanition controls or ad-libitum fed controls. In a later paper, Stoerk _et al_. (1947) reported that antibody response to sheep red blood cell was markedly impaired in pyridoxine deficient rats. Thus, their previous observations about the depressing effect of pyridoxine deficiency on antibody formation was confirmed. Further studies by Stoerk (1948) showed that administering deoxypyridoxine to mice and rats resulted in marked impairment of antibody production. When pyridoxine HCl was given to the animals together with the antagonist, no change in antibodies occurred.

Axelrod *et al*. (1947) observed that the level of circulating antibodies was markedly lowered by pyridoxine deficiency in rats immunized with human red blood cells. Ludovici *et al*. (1951), using immunized rats, reported a reduced serum antibody concentration in pyridoxine deficiency. When pyridoxine was administered to deficient rats, serum antibodies increased and reached normal levels. They suggested that B_6 functions in the process of antibody synthesis. In confirmation of previous findings, Axelrod *et al*. (1961) demonstrated a depressed level of circulating antibodies to diphtheria toxoid in guinea pigs given deoxypyridoxine.

Colucci and Digilio (1957) suggested that the concentration of total serum proteins remained constant in hyperimmunized humans and rabbits inspite of the changes in the levels of circulating antibodies. His report pointed out the possibility of obtaining a change in the level of circulating antibodies, while a constant level of total serum proteins is maintained.

No work has been reported on non-protein-nitrogen (NPN) levels in serum of vitamin B_6 deficient chickens. But data is available on NPN in serum of normal chickens.

Attempts have been made to determine NPN in mammalina species subjected to a B_6 deficiency. Hawkins *et al*. (1946) observed that NPN values in blood of vitamin

B_6 deficient rats were significantly higher than those in control rats. They suggested that this increase in NPN might be due to increased catabolism of amino acids, diminished excretion of urea, to blood concentration, or to a failure in the anabolic processes involving transaminations. The explanation of blood concentration was unlikely, because they found no increase in hemoglobin or in red blood cells. They explained this observation on the basis of a possible failure in transamination

Deutsch and Goodloe (1945) carried out electrophoretic studies on plasma from normal adult White Leghorn females by the moving boundary method. These studies were performed in barbiturate buffer of pH 8.6. The above workers obtained an A/G ratio of 0.7 with 59% globulin and 40.4% albumin. In a later study, the same electrophoretic method was used by Deutsch *et al*. (1949) to study normal and immune serum in young Leghorn females. The percent electrophoretic composition for the normal serum were found to be as follows: Total globulin 54; albumin 46; and gamma globulin 24. Sanders *et al*. (1944) using the same moving boundary method performed electrophoretic analysis of serum from normal and leucosis affected chickens. Determinations were made on 2 serum samples of 4 months old normal White Leghorns. The percent composition of the first sample was found to be 41.7 albumin and 57.4 globulin with an A/G ratio of 0.7, while percent composition of second serum sample was 47.8 albumin and 52.2 globulin with A/G ratio of 0.92.

III. MATERIALS AND METHODS

Experimental Animals

Pre-experimental period. Single Comb White Leghorn day-old chicks were obtained from a commercial hatchery. The chicks were kept for 4 weeks in 5-deck battery brooders equipped with wire floors and thermostatically controlled heating elements. At 1 month of age, they were transferred to 4-deck unheated growing batteries and kept in them till they were 4½ months old.

Throughout the pre-experimental period, the birds were fed *ad-libitum* practical rations commonly used at the Agricultural Research and Education Center (AREC). Six weeks before starting the first experiment, 40 of these birds were leg-banded and transferred to 3 deck individual laying cages. Individual egg production was recorded during the 6 weeks period and used later as a criterion for allocation of birds on treatments.

Experimental period. Experiment I was conducted between November 3, 1965 and February 2, 1966. Thirty, 6 months old layers, were selected and divided into 3 equal groups of similar average body weights, and average egg production. The 3 treatments were assigned at random to the

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cages. The birds were placed in 3 deck individual laying cages, equipped with wire floor, and situated in a room where the temperature ranged between 55° and 65°F.

The experimental design is presented in Table 1. The composition of the experimental diets was similar to that described by Fuller _et al_. (1961). Tables 2 and 3 show the composition of the purified and practical diets used respectively.

Fourteen hours of continuous light was provided per day. Waterers were cleaned twice a day to keep microbial growth at a minimum. Water and feed were supplied _ad-libitum_. Individual feed consumption at 2-week intervals was recorded, and percent egg production calculated. Each bird in the 3 groups was bled at 2-week intervals.

Experiment II was conducted between January 6, 1966 and April 6, 1966. Ten birds, about 9 months old, were divided into 2 equal groups. Each bird was leg-banded and placed in the individual laying cage previously mentioned. The experimental diets were the same as the purified diets of the first experiment. The experimental design is presented in Table 1. Analysis of variance was made according to Snedecor (1956), and all statements regarding significance are based on a 5% level of probability.

Weekly average feed consumption of each bird in group 2 was restricted to that consumed by each bird in

Table 1. Experimental design used.

Treatment	No. of birds	Nature of diet	Vitamin B_6 content
		Experiment I	
Experimental[1]	10	Purified	1.1 mg/kg
Diet control[2]	10	Purified	14.3 mg/kg
Normal control[3]	10	Practical	Biologically adequate
		Experiment II	
Experimental	5	Purified	1.10 mg/kg
Pair-fed Control	5	Purified	13.2 mg/kg

[1] Experimental: Fed purified diet deficient in vitamin B_6.

[2] Diet control: Fed purified diet adequate in vitamin B_6.

[3] Normal control: Fed practical diet adequate in all nutrients.

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Table 2. Purified diet used in experiments I and II.

Ingredients	%
Dextrose	64.35
Isolated soybean protein	20.00
Alpha cel	2.00
Mineral mixture[1]	5.30
Corn oil	3.00
Vitamin mixture[2]	1.10
Choline chloride	0.15
Limestone	4.00
MHA[3]	0.10

[1] Mineral mixture supplied the following per kg of diet: Calcium carbonate 8.8 gm.; calcium phosphate 25.07 gm.; copper sulphate 2.13 gm.; ferric citrate 176.5 mg.; magnesium sulfate 2.65 gm.; manganese sulfate 221 mg.; potassium chloride 6.15 gm.; potassium iodide 9.0 mg.; sodium chloride 3.5 gm.; sodium phosphate dibasic 6.15 gm.; zinc carbonate 115 mg.

[2] Vitamin mixture supplied the following per kg of diet: Vitamin A 9000 I.U.; vitamin D 1000 I,C.U.; alpha tocopherol 50 mg.; ascorbic acid 450 mg.; inositol 50 mg.; choline chloride 750 mg.; riboflavin 10 mg.; menadione 22.5 mg.; P-aminobenzoic acid 50 mg.; niacin 45 mg.; thiamine HCl 10 mg.; calcium pantothenate 30 mg.; biotin 0.2 mg.; folic acid 0.9 mg.; vitamin B_{12} 0.01 mg.
Pyridoxine hydrochloride was added to this mixture in the case of the adequate diets at the rate of 13.2 mg/kg of diet.

[3] Methionine hydroxy analogue calcium salt.

Table 3. Composition of practical diet used in experiment I.

Ingredients	%
Yellow corn	67.50
Soybean meal (44% protein)	24.00
Limestone	5.00
Bone meal	2.75
Salt	0.50
Vitamin and trace mineral mixture*	0.25

* Vitamin and trace mineral mixture supplied the following per kg of diet according to the specifications of the manufacturer: 4400 U.S.P. vitamin A; 1100 I.C.U. vitamin D_3; 5.5 I.U. vitamin E; 3.3 mg riboflavin; 5.5 mg d-pantothenic acid; 27.5 mg niacin; 275 mg choline chloride; 7.92 mcg vitamin B_{12}; 125 mg BHT; 59.8 mg manganese; 26.4 mg zinc; 19.8 mg iron; 1.98 mg copper; 0.99 mg iodine; 0.198 mg cobalt.

group 1. Fourteen hours of continuous light was provided per day, and waterers were cleaned and water changed twice daily. Individual body weights were recorded every week, together with weekly feed consumption. Birds were bled in the middle and at the end of the 3-months test period.

At the termination of the first and second experiments, the birds were slaughtered, dressed, and eviscerated after recording their body weights. The liver, spleen, and gall bladder were removed and weighed immediately. Postmortem examination of the birds was made and incidence of gizzard erosion noted.

Diets

The purified vitamin B_6 adequate diet was supplemented with pure crystalline pyridoxine hydrochloride at the rate of 13.2 mg/kg of diet. Promine R, an isolated soybean protein containing 97% protein on moisture-free basis, and dextrose were used. Alpha cel, a non-nutritive fiber preparation was used to provide bulk in the diet. The composition of Brigg's mineral mixture is shown in Table 2. The choline chloride used was a 70% aqueous solution obtained from Imperial Chemical Industries, Billingham, Durham, England. Methionine hydroxy analogue calcium salt was used to supplement the soybean protein. The vitamin mixture used was a B_6 deficient mixture obtained from Nutrition Biochemicals.

The limestone used in the B_6 deficient diet was heated in an oven in order to destroy any B_6 activity present.

Collection of Serum

Each bird was bled by heart puncture, using a 5 cc sterilized glass syringe and 20 gauge sterilized needle. Bleeding was accomplished by a method described by Hofstad (1950). Five ml of blood were drawn from each bird, transferred directly to sterilized centrifuge labelled tubes, made wet with sterile physiological saline solution, and covered with aluminum foil. Blood in the centrifuge tubes was allowed to clot at room temperature, then centrifuged in an International Centrifuge (I.E.C.) at about 2000 r.p.m. for 15 minutes. Each serum sample was placed in a sterile dry tube labelled with the bird number and covered with a cap. Serum samples were kept in a refrigerator at 4°C for 5 days, then transferred to the freezer. Meanwhile, individual serum samples were analysed for serum glutamic oxalacetic transaminase (SGO-T) activity and nitrogen content. Paper electrophoresis runs were also made during the same period.

Analytical Methods

SGO-T determinations. All chemicals, used in SGO-T analysis, were obtained from Sigma Chemical Company. One ml volume of substrate No. 505-1 containing standardized aspartate

glutarate pH 7.5 was placed in labelled test tubes and kept in a water bath at 37°C. Exactly 0.2 ml of every serum sample was added to the respective tube. The contents of the tubes were mixed by gentle shaking, and replaced into the water bath. Exactly 60 minutes after adding and incubating the serum, 1 ml of sigma color reagent No. 505-2 containing standardized 2,4-dinitrophenyl hydrazine and HCl was added to each tube to stop enzymatic activity and start the color reaction. The tubes were then left at room temperature. Twenty minutes after adding the color reagent, 10 ml of 0.4 N sodium hydroxide No. 505-8 was added for color development, and the tube contents were mixed by inversion. At least 5 minutes after adding sodium hydroxide, the optical density (O.D.) was read at 505 mμ in a Beckman model B spectrophotometer using distilled water as reference. Sigma-Frankel units of S.G.O.-T of the serum was determined from a standard curve where O.D. was plotted against units of S.G.O.-T per ml.

The standard curve was prepared as follows: A calibration standard solution No. 505-10, for preparing standard curves, was added to 6 test tubes in an order of increasing volume, 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml. One ml of sigma color reagent No. 505-2 was added to each tube and the contents mixed by gentle shaking. The tubes were left to stand for 20 minutes at room temperature, after which 10 ml of 0.4 N sodium hydroxide were added to each

tube, and mixing done by inversion. Five minutes after adding the sodium hydroxide, the O.D. of all samples was read at 505 mμ wave length in a Beckman model B spectrophotometer, using distilled water as reference. The standard curve was drawn by plotting the optical densities obtained, versus the corresponding Sigma-Frankel units of SGO-T at 37°C as given in Sigma Bulletin No. 505 (Anonymous, 1960).

Total nitrogen determinations. Nitrogen was determined on the serum samples using the method of Zuazaga (1942) with a minor modification. The procedure consisted of digestion, distillation, and titration. Two spoonfulls of powdered selenium mixture were placed in a micro-kjeldahl flask with added 10 ml of concentrated sulfuric acid. Exactly 0.2 ml of serum was transferred into the bottom of the flask and digested for 3 hours. A blank was prepared for every determination. The digested mixture was then transferred quantitatively into a 50 ml volumetric flask which was filled with distilled water up to volume. Five ml aliquots of the digested mixture were steam distilled into 15 ml of 4% boric acid containing 3 drops of mixed indicator, (5 parts of 0.2% alcoholic bromo cresol green and 1 part of 0.2% alcoholic methyl red solution). Directly after distillation, titration was made with 0.02 N HCl.

Non-protein-nitrogen determinations. The method used in the present study for non-protein-nitrogen determination was developed by Folin and Wu (1919). The preparation of the reagents used was described by Hawk et al. (1954). The protein free filtrate of the serum samples was prepared by the tungstic acid method in the following way: One volume of serum was measured accurately into a dry 125 cc Erlenmeyer flask, followed by 8 volumes of water, and 0.5 volume of 10% sodium tungstate. After mixing by gentle shaking, 0.5 volume of 2/3 N H_2SO_4 was added drop by drop with constant shaking. The flask was closed with a clean rubber stopper and shaken violently. The mixture was then filtered using Whatman filter paper No. 42 ending up with a water clear filtrate. The non-protein-nitrogen of the serum was determined on the protein free filtrate obtained by the tungstic acid method. Three ml aliquots of the serum filtrate were measured into labelled 50 ml volumetric flasks. Glass beads were used to prevent bumping. Two ml of 10 N H_2SO_4 were added to each volumetric flask.

The flasks containing the serum filtrate were placed on hot plates, and boiled until the water vapor was deiven off, and SO_3 fumes filled the flasks. The flasks were allowed to cool then 1 drop of 30% H_2O_2 was added to each and then boiled again for 1 minute. Twenty ml of distilled water were then added to every flask followed by 9 ml of

2.5 N NaOH in order to neutralize the sulfuric acid. Four drops of gum ghatti were added to the flasks which were chilled in ice. Then 5 ml of Nessler's reagent were added to each flask, and made up to volume with distilled water. Five ml of standard solution of ammonium sulphate, containing 0.1 mg N, were placed in a 50 ml volumetric flask, and a similar procedure was followed in preparing this standard. Here also, a blank was prepared for every determination. Optical densities of unknown serum samples and the standard solution were read at 480 mμ in a Beckman model B spectrophotometer.

Paper electrophoresis.

Materials used in making paper electrophoresis runs were as follows:

Buffer solution consisting of 60.5 grams of trishydroxzmethylamino methane (TRIS), 6.0 grams of ethylene diamine tetraacetic acid (EDTA) and 4.6 grams of boric acid was described by Aronsson and Gronwall (1958). These ingredients were dissolved in distilled water and final volume brought up to 1.0 liter. The pH of this buffer was 8.9 and the conductivity 3.0 millimhos.

Amido black stain is prepared by dissolving 1.7 grams in 240 ml rinsing solution.

Rinsing solution is prepared by mixing together 940 ml methanol, 210 ml glacial acetic acid, and 940 ml distilled water.

Method. LKB[1] paper electrophoresis equipment was used throughout the procedure. Four filter paper strips (40 x 410 mm) for type LKB 3276 were used per run. The strips were soaked in the buffer solution for 2 hours and were then allowed to drain by letting them hang for 5 minutes in a vertical position. The electrode vessels were filled with 650 ml of the buffer solution. A glass siphon was used to form a buffer bridge between the 2 electrode vessels and to obtain equilibrium of the buffer levels. The paper strips were placed in the cassette in a plane position. The cassette was covered with its lid and the strips were allowed to equilibrate for 1 hour. A 0.016 ml sample of serum was applied per strip, using the sample applicator. The current used for 16 hours run corresponds to 0.25 mA/10 mm width of paper strips. The current was set on 4.0 mA, for 16 hours. Then the electricity was put off and the paper strips were taken out and immediately dried in an oven at 225°F for about 30 minutes. The paper strips were stained and washed as follows. Every group of 4 paper strips was immersed twice in 50 ml amido black solution, each time for 10 minutes. They were then washed with 100 ml rinsing solution 7 times of 20 minutes each. After the seventh washing, the paper strips were dried in the oven, and then scanned in a Spinco model R analytrol.

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1 LKB - producter AB; P. O. Box 76, Stockholm - Bromma 1, Sweden

IV. RESULTS AND DISCUSSION

Body Weight, Organ Weight, and Egg Production

Chickens fed the pyridoxine deficient diet developed several symptoms of vitamin B_6 deficiency. These were reduced feed intake, loss in body weight, cessation of egg production, hyperexcitability and droopiness. Moderate reduction in feed consumption was observed at the end of the first 2 weeks of the experimental period. Hyperexcitability and droopiness appeared on the 5th - 6th week of the experimental period and increased in severity as the experiment progressed.

Data on body weight are presented in Table 4. The average body weights per bird in each group were relatively equal at the start of each experiment. In the course of experiment I, there was appreciable loss in average body weight of the birds fed the pyridoxine deficient diet, while the control birds gained slightly. Statistical analysis of body weight at the 12th week of the experimental period presented in Table 12 indicates that reduction in body weight due to B_6 deficiency is statistically significant. This was also the case in experiment II as shown in Table 13. In experiment I, the average body weight of birds fed purified B_6-adequate diets were

Table 4. Effect of vitamin B_6 deficiency on body weight (gm) in mature female chickens.

Period	Experiment I			Experiment II	
	Experimental	Diet control	Normal control	Experimental	Pair-fed control
Start	1529 ± 51*	1485 ± 57	1491 ± 54	1755 ± 54	1730 ± 80
2nd week	1326 ± 47	1498 ± 54	1512 ± 61	1541 ± 60	1549 ± 54
4th week	1423 ± 32	1494 ± 52	1546 ± 60	1347 ± 38	1546 ± 32
6th week	1104 ± 34	1455 ± 52	1516 ± 55	1231 ± 13	1461 ± 42
8th week	1051 ± 42	1514 ± 54	1558 ± 65	1178 ± 33	1466 ± 40
10th week	1005 ± 40	1549 ± 53	1546 ± 60	1133 ± 29	1402 ± 43
12th week	993 ± 35	1523 ± 55	1582 ± 57	1055 ± 33	1302 ± 58

* Mean ± Standard Error (S.E.).

comparable to those fed the practical diet which indicates the adequacy of the purified diet supplemented with vitamin B_6.

Body weight in the pair-fed group of experiment II was not reduced to the same extent as that of the experimental. This indicates that body weight loss was not due only to reduced feed intake but also to vitamin B_6 deficiency per se.

A record of feed consumption per bird at 2-week intervals is presented in Table 5. The feed consumption of the vitamin B_6 deficient group was markedly lowered due to pyridoxine deficiency. This was observed as early as the first 2 weeks of the experimental period. The fact that feed intake of the 2 control groups was not equal may be attributed to differences in palatability of the feed or energy levels; the purified diet being less palatable and higher in energy. Weekly feed intake of a pair-fed adequate bird in experiment II, was restricted to 400-520 grams of feed which was the actual amount consumed by an experimental bird.

Data on egg production is presented in Table 6. The sharp drop in egg production in the experimental group, and then the complete cessation of production on the 4th - 5th week are indicative of the development of the B_6 deficiency state. Egg production in birds fed the B_6 adequate purified

Table 5. Effect of vitamin B_6 deficiency on feed consumption (gm) of mature female chickens - Experiment I.

Period	Experimental	Diet control	Normal control
0 - 2 weeks	876	1050	1323
2 - 4 weeks	620	1300	1315
4 - 6 weeks	721	1264	1816
6 - 8 weeks	830	1285	1200
8 - 10 weeks	688	1297	1652
10 - 12 weeks	688	1174	1752

Table 6. Effect of vitamin B_6 deficiency on percent egg production - Experiment I.

Period	Experimental	Diet control	Normal control
Start	83 ± 3.1*	84 ± 3.6	83 ± 3.2
2nd week	59 ± 6.2	43 ± 7.0	69 ± 10.0
4th week	1.4 ± 1.4	34 ± 9.8	67 ± 8.3
6th week	-	47 ± 4.6	72 ± 7.5
8th week	-	43 ± 4.3	77 ± 3.9
10th week	-	30 ± 9.3	66 ± 8.1
12th week	-	42 ± 8.9	78 ± 3.8

* Mean ± S.E.

diet was lower than that in the group fed the practical diet. This may be due to lower feed consumption of these birds or partly due to the marginality of this ration in a factor important for egg production. This observation is in agreement with the report of Fuller et al. (1961) who observed a drop in egg production in birds receiving a purified B_6-adequate diet. Restriction of feed intake in experiment II resulted in cessation of egg production in the pair-fed B_6-adequate group on the 2nd - 3rd week of the experimental period. However, 2 birds in this group were able to produce a total of 7 eggs during the last 2 months of the experimental period. Despite feed restriction, these birds received adequate amounts of nutrients sufficient to meet their body maintenance requirements and lay few eggs.

Table 7 shows the average organ weights of all groups in experiments I and II. A slight increase in percent liver weight (g/100 g body weight) was observed in the experimental birds in comparison to the diet control birds. This increase was significant in experiment II, but not in experiment I. This may indicate hypertrophy of the liver caused by B_6 deficiency. Hsu and Kawin (1962) found that B_6 deficiency results in a true hypertrophy of the liver, kidney and adrenal gland, and an atrophy of the thymus in rats. Cambridge (1956) reported

Table 7. Influence of vitamin B_6 deficiency on organ weight (gm).

Treatments	Experiment I			Experiment II	
	Experimental	Diet control	Normal control	Experimental	Pair-fed control
Body weight	992 ± 42*	1524 ± 58	1571 ± 72	1055 ± 33	1302 ± 58
Liver weight	21.8 ± 2.71	28.7 ± 2.33	37.9 ± 1.9	21.5 ± 1.65	21.7 ± 1.06
% liver weight	2.18 ± 0.21	1.89 ± 0.14	2.40 ± 0.12	2.00 ± 0.12	1.67 ± 0.06
Spleen weight	0.99 ± 0.11	1.19 ± 0.11	1.71 ± 0.45	0.83 ± 0.04	1.74 ± 0.26
% spleen weight	0.098 ± 0.008	0.077 ± 0.005	0.110 ± 0.031	0.079 ± 0.003	0.130 ± 0.014
Gall bladder	0.78 ± 0.13	1.40 ± 0.01	1.63 ± 0.14	1.00 ± 0.23	1.26 ± 0.28
% gall bladder weight	0.079 ± 0.005	0.093 ± 0.007	0.110 ± 0.010	0.097 ± 0.020	0.096 ± 0.019

* Mean ± S.E.

similar observations also in rats. Our results do not fully support hypertrophy of the liver as a result of B_6 deficiency because of the variation encountered. They indicate however, that liver weight in the B_6 deficient birds did not decrease at the same rate as their body weights. Absolute liver weights of the B_6- deficient groups are all lower than the B_6 adequate groups. It is interesting to note that liver weights of the normal control are much higher than those of the diet control although they have similar body weights. This may reflect the greater need for liver tissue in birds receiving a practical diet versus those receiving a purified diet.

SGO-T

Data presented in Table 8 shows SGO-T values determined on 5 collections of serum in experiment I. The first determination was performed 26 days after placing the birds on the experimental diets, while the remaining 4 determinations were made at 2-week intervals. Analysis of variance of this data presented in Table 12 shows a statistically significant difference between treatments. The reduction of SGO-T activity in B_6 deficiency has previously been reported by several workers. Goswami and Robblee (1958) showed considerable reduction in blood GOT activity of chicks fed a B_6 deficient diet. Comparing

Table 8. Effect of vitamin B_6 deficiency on serum glutamic oxalacetic transaminase activity (U/ml).

Period	Experimental	Diet control	Normal control
	Experiment I		
4th week	134 ± 2.9*	167 ± 6.5	163 ± 15.8
6th week	150 ± 8.9	178 ± 9.6	179 ± 7.8
8th week	133 ± 4.2	189 ± 10.2	185 ± 20.5
10th week	156 ± 7.6	220 ± 8.5	210 ± 15.8
12th week	142 ± 12.9	213 ± 24.3	208 ± 22.5
Average	143 ± 4.5	194 ± 10.0	189 ± 8.9
	Experiment II		
	Experimental	Pair-fed control	
6th week	118 ± 5.4*	163 ± 14.7	
10th week	113 ± 9.0	159 ± 10.9	
12th week	160 ± 23.5	196 ± 21.8	
Average	130 ± 14.8	173 ± 11.7	

*. Mean ± S.E.

the SGO-T levels of the experimental and control groups, it is observed that the depression of SGO-T level in the experimental group is about 22-25 percent of the control groups. This agrees with the report of Brin et al. (1960) who found a moderate reduction of plasma GOT in the adult rats (22%) whereas a marked depression of plasma GOT was noted in young rats (63%). Results obtained in the second experiment confirm the previous results as shown in Table 8.

Serum Total Nitrogen and Non-Protein-Nitrogen

Data on total nitrogen are presented in Tables 9 and 10. No statistically significant differences were found in total nitrogen content of serum samples obtained from the experimental and control groups in both experiments.

Results of experiment II, presented in Table 10, show that NPN values of the experimental birds were significantly higher than those of the pair-fed group. This trend was also observed in experiment I, but differences were not found to be statistically significant. This trend is supported by Hawkins et al. (1946) who observed that NPN values in blood of vitamin B_6 deficient rats were significantly higher than those in the control rats. Upon examining these results, the protein nitrogen of the serum

Table 9. Effect of vitamin B_6 deficiency on serum non-protein-nitrogen (mg/100 ml) and total nitrogen (gm/100 ml) of mature female chickens - Experiment I.

Period	Experimental		Diet control		Normal control	
	Total N	NPN	Total N	NPN	Total N	NPN
5th week	0.57 ± 0.048*	18.4 ± 1.5	0.57 ± 0.040	19.3 ± 2.1	0.60 ± 0.020	17.9 ± 0.8
7th week	0.64 ± 0.030	18.6 ± 1.4	0.63 ± 0.029	15.4 ± 2.0	0.65 ± 0.018	17.5 ± 2.2
9th week	0.67 ± 0.015	19.2 ± 0.8	0.64 ± 0.027	18.3 ± 1.5	0.68 ± 0.015	17.5 ± 1.9
11th week	0.70 ± 0.027	18.6 ± 1.5	0.66 ± 0.014	17.4 ± 0.7	0.67 ± 0.018	12.5 ± 0.8
Average	0.64 ± 0.030	18.7 ± 1.3	0.62 ± 0.027	17.6 ± 1.6	0.65 ± 0.017	16.3 ± 1.4

* Each figure is the mean ± S.E. of 7 determinations except for the first period of diet control where 8 determinations were made.

Table 10. Influence of vitamin B_6 deficiency on serum non-protein-nitrogen (mg/100 ml) and total nitrogen (gm/100 ml) of mature female chickens - Experiment II.

	Experimental		Pair-fed control	
Period	Total N	NPN	Total N	NPN
6th week	0.68 ± 0.011*	18.0 ± 2.4	0.66 ± 0.018	15.7 ± 0.4
12th week	0.69 ± 0.010	20.0 ± 1.5	0.70 ± 0.030	18.0 ± 0.8
Average	0.68 ± 0.01	19.4 ± 1.9	0.68 ± 0.02	16.9 ± 0.6

* Each figure is the mean ± S.E. of 5 determinations.

appears to be relatively similar in the experimental and control groups. Moreover, these results indicate that total serum protein concentrations are not influenced by vitamin B_6 deficiency. Similar observations were reported by Beaton _et al_. (1953) who observed no differences in the concentrations of serum proteins between B_6 deficient and control rats. Vilter _et al_. (1953) found also no changes in concentrations of total serum proteins of human patients as a result of the B_6 deficiency.

Electrophoretic Serum Protein Fractions

Figures 1 and 2 show electrophoretic patterns of 2 serum samples collected at the 5th week and end of the first experiment from 1 bird representative of the B_6 deficient group. The electrophoretic patterns of serum obtained at the 5th week and end of the first experiment from a control bird on the purified B_6 adequate diet are shown in Figures 3 and 4, respectively. The nature of the pattern appears to be similar in the deficient and control groups. These patterns show sharp and tall albumin peaks. Variations among the electrophoretic patterns were noted in all treatments with respect to separation of the globulin fractions. Figure 2 shows distinct peaks of beta and gamma globulins, while Figure 4 does not show complete separation of globulins.

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Data on percent albumin and percent globulin are presented in Table 11. In experiment I, the control groups had lower percent albumin and higher percent globulin than the experimental group. This was not very apparent in experiment II where a pair-fed control was used. The albumin/globulin ratios (A/G) were obtained by calculating the areas under the curve for albumin and globulin from the analytrol graphic chart. Data on A/G ratios are presented in Table 11. Statistical analysis of A/G ratios showed no significant differences in either experiment.

Our electrophoretic results are in agreement with those obtained by Sanders _et al_. (1944) and Deutsch and Goodloe (1945) in which the moving boundary method was used.

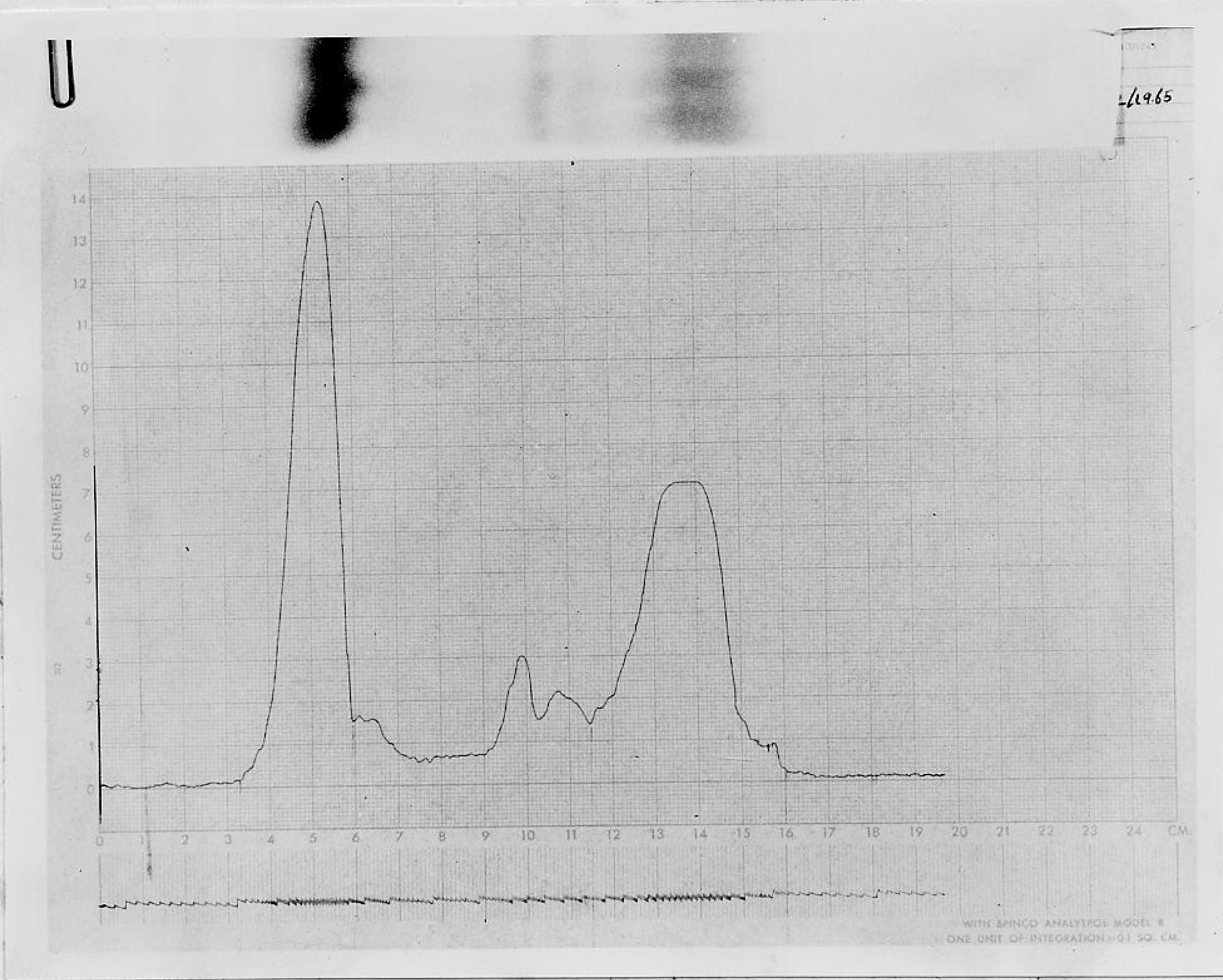

Figure 1. Electrophoretic patterns obtained from serum of deficient bird at 5th week of experiment I.

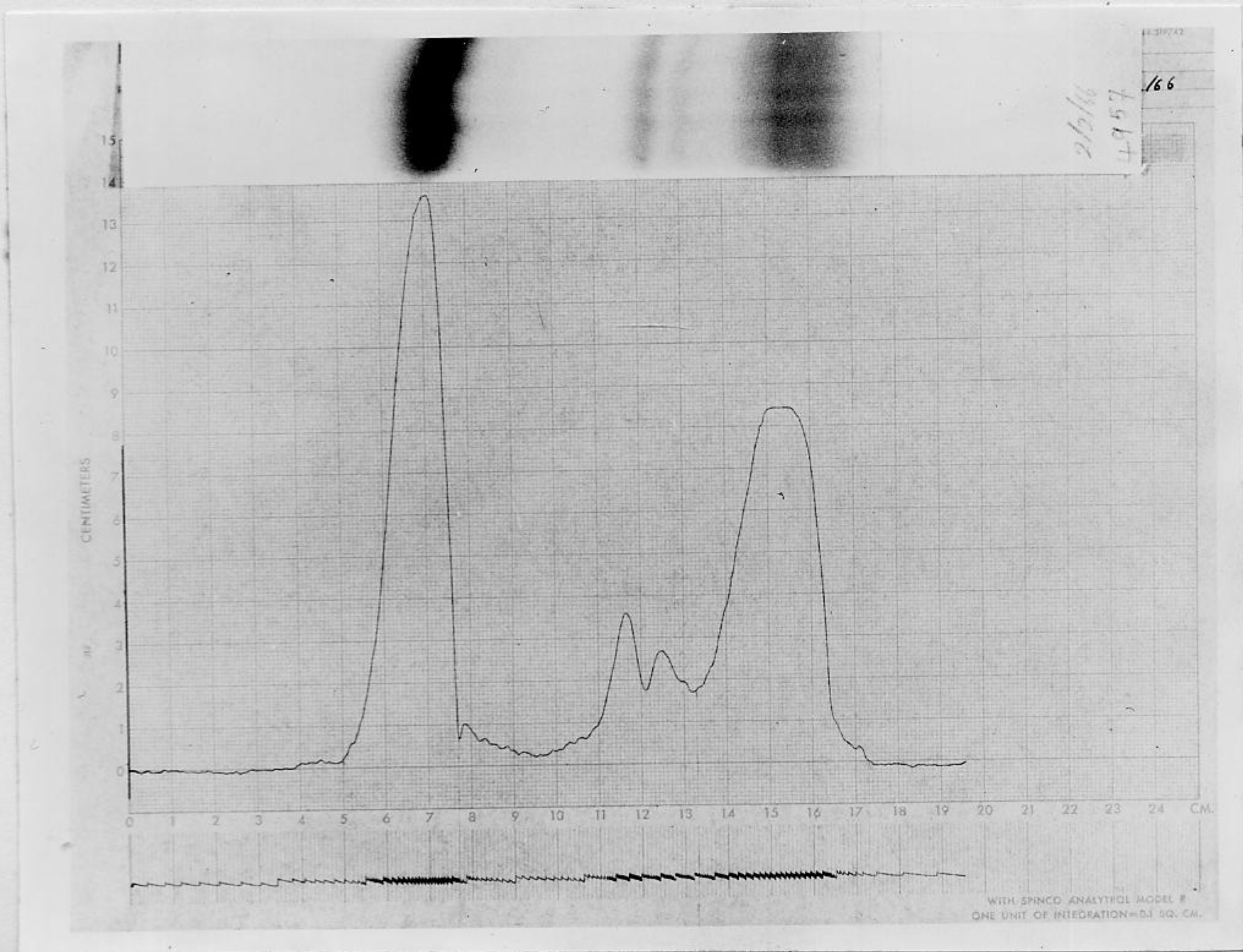

Figure 2. Electrophoretic patterns obtained from serum of the same bird at 12th week of experiment I.

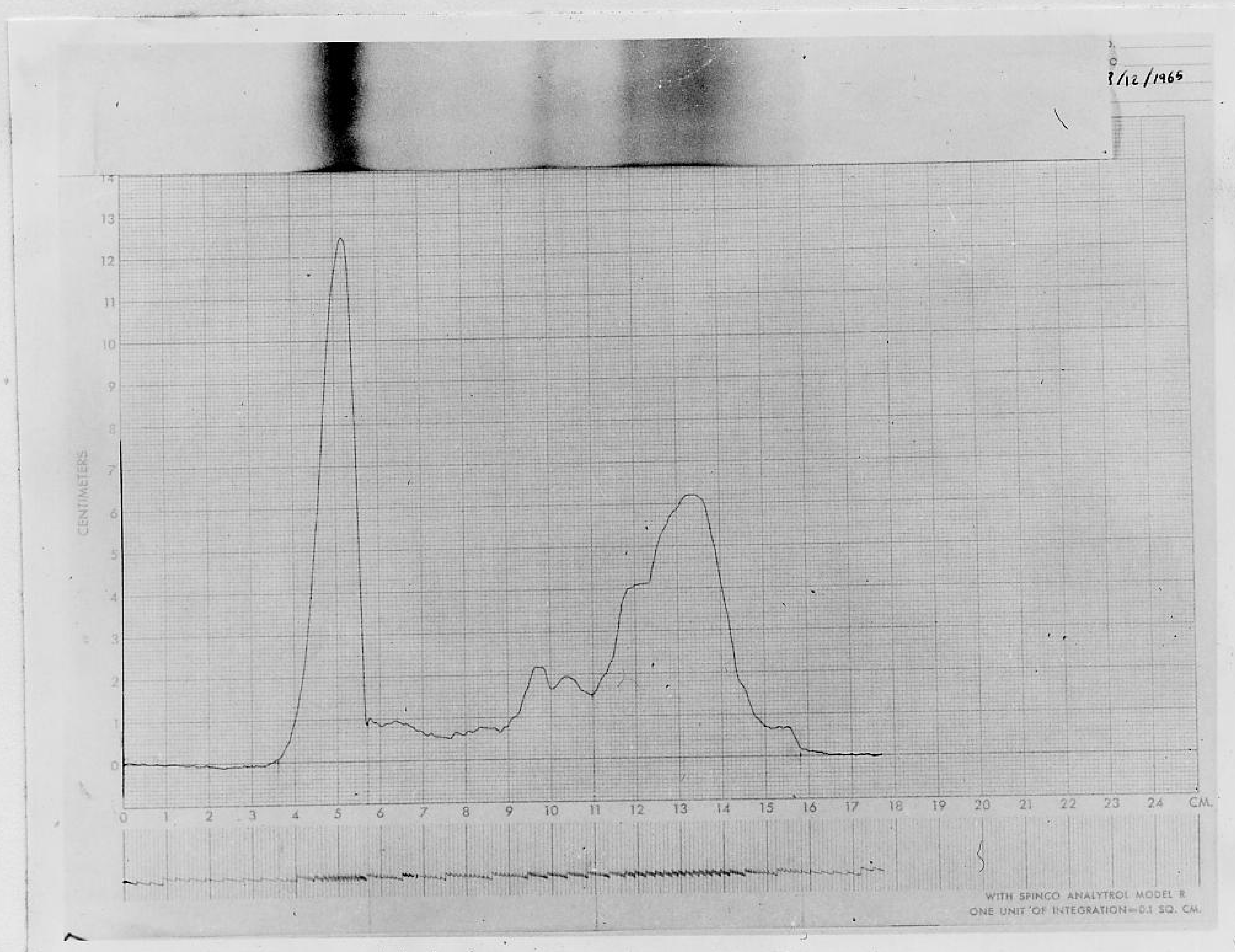

Figure 3. Electrophoretic patterns obtained from serum of B_6 adequate bird at 5th week of experiment I.

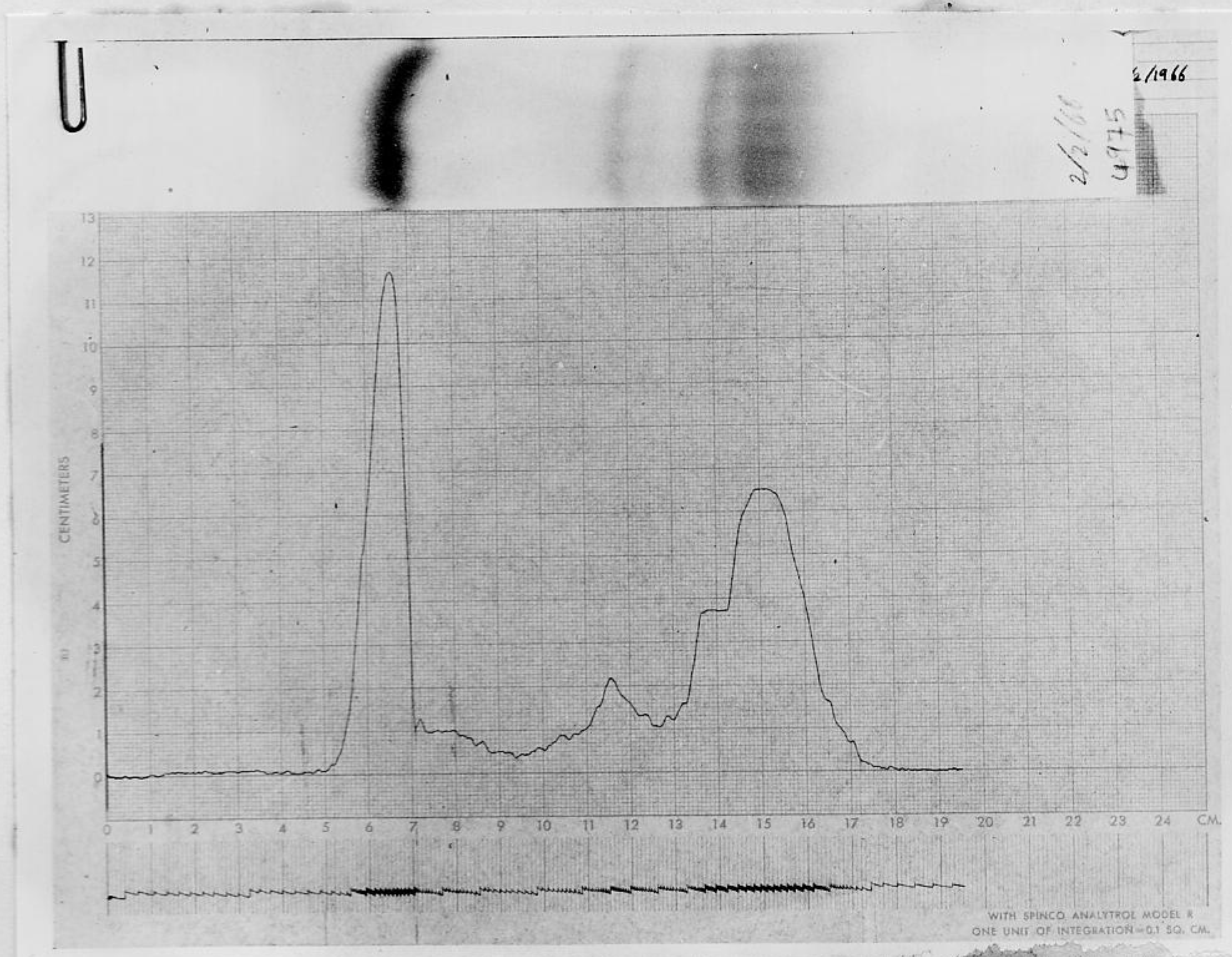

Figure 4. Electrophoretic patterns obtained from serum of the same B_6 adequate bird at 12th week of experiment I.

Table 11. Effect of vitamin B_6 deficiency on serum protein fractions of mature female chickens.

Experiment I - 32 days after start of experiment					End of experiment			
	% Albumin	% Globulin	% γ Globulin	A/G	% Albumin	% Globulin	% γ Globulin	A/G
Experimental	44.8±3.26*	55.2±3.2	35.7±3.3	0.81±0.09	44.2±1.39	55.8±1.3	35.5±2.2	0.79±0.04
Diet control	40.8±3.50	59.2±3.5	38.4±2.9	0.69±0.10	36.0±1.42	64.0±1.4	39.7±2.3	0.57±0.03
Normal control	40.6±0.81	59.4±0.8	37.9±1.0	0.68±0.02	39.7±1.71	60.3±1.7	39.0±1.9	0.66±0.04
Experiment II - 45 days after start of experiment					**End of experiment**			
Experimental	39.7±1.8	60.3±1.8	38.3±0.3	0.66±0.05	45.2±1.2	54.7±1.2	36.2±1.3	0.82±0.04
Pair-fed control	40.7±0.9	59.2±1.6	34.9±2.8	0.69±0.02	43.2±3.6	56.8±2.4	41.2±3.0	0.76±0.07

* Mean ± S.E.

Table 12. Analysis of variance for body weights, SGO-T, percent liver weight, NPN, total N, and A/G ratio - Experiment I.

Source of variation	d.f.	M.S.		d.f.	M.S.	d.f.	M.S.		d.f.	M.S.
		Body wt.	% liver wt.		SGO-T		NPN	Total N		A/G
Replicates[1] or periods	9	27142	0.164	4	794[3]	3	3.7	0.0055[3]	1	0.004
Treatments	2	105099[2]	0.705	2	3896[3]	2	5.0	0.00075	2	0.0158
Error	18	25308	4.143	8	107	6	3.0	0.000184	2	0.0018

[1] Periods were used as replicates for determining M.S. of SGO-T, NPN, total N, and A/G ratio.

[2] Significant at the 5% level of probability.

[3] Significant at the 1% level of probability.

Table 13. Analysis of variance for body weights, SGO-T, percent liver weight, NPN, total N, and A/G ratio - Experiment II.

Source of variation	d.f.	M.S.		d.f.	M.S.	d.f.	M.S.		d.f.	M.S.
		Body wt.	% liver wt.		SGO-T		NPN	Total N		A/G
Replicates or periods[1]	4	9982	0.06	2	1089[3]	1	6.30[2]	0.0006	1	0.0132
Treatments	1	153016[2]	0.33[2]	1	2676[3]	1	6.30[2]	0.0002	1	0.0003
Error	4	12463	0.034	2	17.5	1	0.0081	0.0001	1	0.0020

[1] Periods were used as replicates for determining M.S. of SGO-T, NPN, total N and A/G ratio.

[2] Significant at the 5% level of probability.

[3] Significant at the 1% level of probability.

V. SUMMARY AND CONCLUSIONS

Two experiments were conducted to study the effect of vitamin B_6 deficiency on serum proteins in White Leghorn female chickens. In the first experiment, 3 groups of 10 birds each were used. The experimental group was fed a highly purified B_6-deficient diet, while the 2 control groups were fed either a purified B_6-adequate diet or a practical diet. All birds were 6 months old and laying at the rate of 80% when the experiment was started. In the second experiment, 2 groups of 5 birds each were pair-fed a purified diet with and without B_6 supplementation.

Data on body weight, feed consumption, egg production and organ weight were collected. Serum was analyzed for SGO-T activity, total nitrogen content, and non-protein-nitrogen. Paper electrophoresis technique was used to study possible changes in serum protein fractions.

Vitamin B_6 deficiency induced in mature female chickens by feeding a pyridoxine deficient diet was manifested by loss in body weight, cessation of egg production, drop in feed consumption, and reduction in SGO-T activity. By using a pair-fed control, loss in body weight was shown to be due to reduced feed intake as well as to vitamin B_6 deficiency per se. Complete cessation of egg production

was observed in B_6-deficient birds 4-5 weeks after placing them on the experimental diet. There was an increase in liver weight expressed in grams per 100 grams of body weight as a result of pyridoxine deficiency. This increase was statistically significant in experiment II only.

A statistically significant reduction in SGO-T activity of the B_6 deficient birds was observed in both experiments. No significant changes were detected in serum total nitrogen in either experiment as a result of pyridoxine deficiency. A slight increase in non-protein-nitrogen values was found in B_6 deficient birds in both experiments. This increase was statistically significant in experiment II, but not in experiment I.

Statistical analysis of the albumin/globulin ratio showed no significant differences between treatments in either experiment.

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