FURTHER STUDIES ON SUPPLEMENTATION OF ALL-VEGETABLE CHICK RATIONS

FURTHER STUDIES ON SUPPLEMENTATION OF ALL-VEGETABLE CHICK RATIONS

By

JEAN HUSTON

Bachelor of Science

Purdue University

Lafayette, Indiana

1946

Submitted to the Department of Household Science

Oklahoma Agricultural and Mechanical College

In Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

1948

APPROVED BY:

Chairman, Thesis Committee

Member of the Thesis Committee

Head of the Department

Dean of the Graduate School

ACKNOWLEDGMENT

The author wishes to express her sincere appreciation to Dr. Robert MacVicar and Mr. R. H. Thayer under whose direction this investigation has been conducted.

Appreciation is expressed to the Oklahoma Agricultural Experiment Station for financial support of this research, and to the Poultry Department for providing the experimental material.

Acknowledgment is also made to Dr. Daisy I. Purdy for helpful guidance during the entire course of the author's graduate program.

TABLE OF CONTENTS

INTRODUCTION

For several years research workers have been studying a heretofore unidentified growth factor for chicks and rats, the presence of which has been noted in fish preparations, liver, distillers' dried solubles, and certain by-products of the canning and packing industries. When these products were incorporated into certain rations, a marked acceleration in growth rate took place, which was apparently unrelated to the protein content of the products.

This project was undertaken to study further the growth promoting activity of two of the known sources of this factor, fish solubles and liver. Previous work had been done at this station on the separation of the active material in fish solubles, and this study presents results of continued fractionation of this product and of liver.

REVIEW OF LITERATURE

Wilgus and Zander (30) found that soybean oil meal used as the sole source of protein supplement in an all-vegetable ration did not produce satisfactory growth in chicks. Adding as little as 1.25% of meat and bone scrap as a supplement to the ration improved growth, and 2.5% produced optimum rates of gain. Likewise, Berry et al (1) reported that rations containing 32% soybean oil meal produced a decreased rate of growth when compared with rations containing 20% soybean oil meal, 12% corn gluten meal, and 3% meat and bone scrap. They also obtained highly significant chick growth increases when 2% or 4% fish press water was added to an all-vegetable ration. Brant and Carver (5) reported that when soybean oil meal was the sole protein source in the ration, chicks showed slower gains in weight, and the efficiency of ration utilization was less than when fish meal or bone scrap was added to the ration. These differences were not observed during the developing and laying periods. Hammond and Titus (10) found that when soybean oil meal was used at a 35% level as the protein supplement in the diet, supplementation with vitamins and minerals was required in order to secure rapid growth in chicks. Addition of sardine meal at a 2% level to this soybean oil meal diet produced gains in growth superior to a diet containing meat scrap and dried skim milk. Heuser (11) reported that soybean oil meal as the only supplementary protein in an all-vegetable ration produced heavier chicks at eight weeks than peanut oil meal or wheat germ meal, which in turn were better than cottonseed oil meal and corn gluten meal. Inclusion of 3% fish meal increased response in every case.

The workers concluded that the effect of the animal protein was not due primarily to the added amino acids.

A great deal of controversy has taken place concerning the possibility of correcting the deficiencies of the all-vegetable ration by addition of the known factors of the vitamin B complex. Patton et al (22) supported by many other workers, found that on a diet composed largely of ground corn, soybean oil meal, minerals, and well supplied with the known vitamins, chicks needed an animal protein supplement for maximum growth. Mishler et al (18) reported that a 55% corn and 40% soybean oil meal ration contained adequate amounts of biotin, inositol, para-amino benzoic acid, and vitamins E and K. Supplementing a diet with riboflavin, pantothenic acid, nicotinic acid, pyridoxine, vitamins A and D, and minerals, produced good growth. They concluded, therefore, that animal protein supplements were not essential for good chick growth. Marvel et al (16) showed that addition of distillers' dried solubles to a vegetable protein ration supplemented with minerals and vitamins produced growth equal to that of a ration supplemented with meat scrap and dried buttermilk. They concluded that animal proteins in themselves were not essential for good growth in young chicks.

Novak et al (21) reported that distillers' dried solubles and condensed fish solubles both contained an unknown growth factor or factors necessary for growth of chicks. Cravens et al (6) found that a diet composed of yellow corn, wheat by-products, meat scraps, soybean oil meal, minerals, riboflavin, and fish oil was unsatisfactory as a diet for growing chicks. Addition of 5%

alfalfa leaf meal failed to give significant growth improvement. A slight improvement was noted with the addition of 5% dried skim milk. Condensed fish press water or ground fish viscera was highly effective in producing growth gains; a combination of dried skim milk and fish press water resulted in the greatest growth stimulus. Grau (8) reported, in feeding experiments using fish solubles at a 5% level in a diet of soybean oil meal and alfalfa, supplemented with minerals and vitamins, that the growth of chicks was greatly stimulated. The protein content of the fish solubles, he felt, was not responsible for the stimulatory action. Mishler et al (17) obtained best growth using fish solubles at a 1.5% level in a ration containing 36% soybean oil meal, 58% corn, and mineral and vitamin supplements. Lassen and Bacon (13) found that condensed fish solubles could be incorporated into poultry rations in amounts as high as 13%. Above this amount the high mineral content counteracted the growth promotion. Bird et al (4) and Bethke et al (2) showed that sardine fish meal and condensed fish solubles increased hatchability. They suggested that the growth factor was transmitted from the hen through the egg to the chick. In confirmation of this view McGinnis and Carver (14) reported that the need for the factor in the chick was markedly influenced by the diet fed the hen. High mortality was observed in chicks from hens fed soybean oil meal supplemented by 1.7% fish meal, while chicks from breeder hens on diets containing 4.5% fish meal showed maximum growth and livability.

Emerson (7) using 50% and 66% alcohol fractions of fish solubles at a 3% level in the feed, found the activity to be in the alcohol insoluble fractions. Nichol et al (19) reported a

significant growth response in chick rations containing an ethanol insoluble fraction of fish solubles added to the basal ration at a level equivalent to 3% of the fish solubles. Reticulogen, an anti-pernicious anemia factor injected at a level of 0.05 cc., produced a growth stimulation equal to that of the ration containing fish solubles. In studies with purified rations, these workers (23) reported a similar growth response. They found the unidentified growth factor in fish solubles to be insoluble in 95% ethanol, acetone, and ether, and soluble in water and 70% methanol, stating that the higher the concentration of alcohol, the less soluble the factor was.

Hammond (9) and Whitson et al (29) found that cow manure produced a growth stimulus when added to a ration supplemented by vitamins and minerals, and Whitson further reported that the growth promoting factor was not one of the known vitamins. Rubin and Bird (25) observed that the growth factor in cow manure was not identical with the previously described growth factors. They prepared an ethanol soluble extract from the manure that was effective in promoting chick growth. Bird et al (3) later reported this growth factor in cow manure to be soluble in water at pH 3.0 if the protein were removed, soluble in 80% acetone, stable to autoclaving for two hours at a neutral pH, but readily destroyed by autoclaving it one hour at an acid pH. Some destruction of this growth factor took place when the manure was dried or was allowed to stand in a slightly alkaline solution. The workers developed a 1-2 week assay procedure using chicks, previously fed a diet deficient in the essential growth factor.

Rubin et al (26) found that a chick growth factor was also present in urine free hen droppings, and concluded that the growth substance must have been synthesized by the hen since it was not in the hen's diet. It was not found in the droppings of growing chicks three to six weeks old.

McGinnis et al (15) reported an unidentified growth factor in liver. The growth promoting factor was found in an ethanol soluble fraction. They found that this factor was uninfluenced by the type of protein in the diet. Methionine, for instance, didn't replace the factor, but gave additional growth response. The factor was destroyed by oxidation, was not absorbed by Darco at a pH of 5, 4, 3, or 2, and was insoluble in acetone. The active fraction was dialyzable through a cellophane membrane. Optimum growth was obtained in grain rations using as little as three milligrams of different concentrates per 100 grams of feed. Johnson et al (12) reported a factor present in casein and liver meal essential for chick growth. It was distinct from vitamin A and the known members of the vitamin B complex. It was soluble in ether and in ethanol, and was thermostable. Zucker and Zucker (31) in studies with rats found an unidentified growth factor, which they named "Zoopherin" present in 1:20 liver powder, fish solubles, and crude casein respectively. They reported the factor to be soluble in water, dilute acid, alkali, moderately soluble in 95% alcohol, and insoluble in ether. It was stable to heat, light, and air. Novak and Hauge (20) likewise reported a growth factor for rats, which they called vitamin B_{13}, obtained from distillers' dried solubles, rice polishings concentrate,

and liver extract in a highly purified state. 10γ of this substance produced maximum growth in the rats, while 2γ gave definite growth stimulation. It was stable to heat, acid, and alkali, and soluble in water, acetone, chloroform, ethanol, ethyl ether, and benzene. It was precipitated by phosphotungstic acid and lead acetate, and absorbed from acid solution on Florisil, Lloyd's reagent, norit, and Decalso. The solubility of the substance in chloroform and ether indicated that it was not identical with the cow manure factor.

Within the past few months, Rickes et al (24) have reported the isolation in crystalline form of a compound which they call vitamin B_{12}. This compound, isolated from liver, has produced positive hematological responses at doses of a few micrograms in cases of Addisonian pernicious anemia. (West, 28) It seems likely that vitamin B_{12} may be in part at least, responsible for the chick growth promoting activity of the highly potent anti-pernicious anemia (APA) preparations. Vitamin B_{12} appears to be the growth factor required by Lactobacillus lactis Dorner (LLD factor). Shorb (27) found that "Vitamin B_{12} when assayed for LLD activity was found to be wholly or partially responsible for the growth activity." The LLD factor activity occurs in fairly high amounts in cow manure, fish meal, pancreatin, papain, egg white and egg yolk, and in lesser amounts in alcoholic extracts of whey, soybean oil meal, gelatin, and zein.

EXPERIMENTAL

General

In all of these studies day-old male chicks were used. 14 chicks were included in each lot in trial 1, and in all subsequent trials 12 chicks were used in each lot, with one exception in trial 4. Chicks were a mixture of White Plymouth Rocks, New Hampshires, and Dominant White crosses. They were wing-banded and distributed at random through the lots. Trials were carried for a period of four weeks each.

The chicks were housed in a thermostatically controlled, electrically heated battery brooder, maintained at suitable temperature throughout the experiments. Food and water were provided ad lib, and the chicks were weighed at weekly intervals. Weight records for the individual chicks were kept, but the weights recorded in this paper were the average weekly gain obtained for each entire lot.

Chemical Fractionation of Fish Solubles

In a previous study on chick growth made at this experiment station (7), it was concluded that chicks fed an all-vegetable ration supplemented with synthetic B-complex vitamins showed as great a gain in weight during 4 and 6 weeks periods as did chicks fed a practical poultry ration containing animal protein. A study of the addition of the B-complex vitamins showed maximum growth only when all the vitamins were included in the ration. The most significant drop in growth was noted when choline was

omitted. When the all-vegetable ration was supplemented with the B-complex vitamins and 3% fish solubles, growth rates of the chicks greatly increased. Chemical fractionation of the fish solubles using 50% and 66% alcohol concentrations, showed the growth activity to be in the fraction soluble in 66% and insoluble in 95% alcohol.

The fish solubles used in these experiments was what is commonly known as fish press water, consisting of the body moisture, gastric juices, tissue particles, etc. removed from freshly pressed sardine fish oil. This material was then reduced to a 50% solids consistency through a series of vacuum evaporations. The protein content of the fish solubles used was approximately 33%.

Chemical fractionation of the growth active material in the fish solubles was begun, using various concentrations of alcohol, then precipitating agents.

Since growth activity for chicks has also been noted using various liver preparations it was thought advisable to compare the growth obtained using liver fractions with that of the fish soluble fractions. Certain liver fractions which had been prepared from a commercial 1:20 liver powder (Wilson) were available in the laboratory. Methods of fractionation for the liver were similar to those of comparable fish soluble fractions, the preparation of which will be discussed in detail.

The fractions were tested for growth promoting activity by adding them to a corn and soybean oil meal ration, supplemented

with B-complex vitamins and minerals. Composition of the basal ration and the B-complex vitamin supplements is shown in Table I. In all cases fish solubles, as such, were added to the ration at a 3% level, while all fractions made from this product were added to the ration at a level of 4% of the original material, 1% being allowed for losses during processing. 1:20 liver powder and liver fractions were added at levels as designated.

Trial 1

It was felt desireble to first try to duplicate the results obtained in the previous study with the fish soluble fractionation, then to attempt to carry the fractionation through further stages of purification.

Lots 1, 2, and 4 served as controls. Lot 1 was fed the basal ration, lot 2 the basal ration supplemented with B vitamins, and lot 4 the basal ration supplemented with B vitamins and fish solubles. Since it had been noted previously that omission of choline from the vitamin B-complex supplements produced the most serious reduction in chick growth, it was considered worthwhile to determine whether choline alone would adequately supplement the ration. Therefore, in lot 3 choline was the only vitamin supplement.

Fish soluble fractions were prepared in the following manner: 2000 gms. of fish solubles and 3340 cc. of 95% alcohol were mixed together, making a 66% alcohol concentration. This mixture was stirred until homogeneous using a mechanical stirrer, then allowed

TABLE I.

BASAL RATION

Ingredient	Per Cent
Ground yellow corn	57.0
Soybean oil meal	39.0
Calcium carbonate	1.0
Steamed bonemeal	2.0
Salt	1.0
TOTAL	100.0

The ration was supplemented with the following vitamins per 100 pounds of feed: Vitamin A and D feed oil, 115 gms.; Thiamine, 90 mg.; Inositol, 1 gm.; Para-amino benzoic acid, 500 mg.; Pantothenic acid, 500 mg.; Pyridoxine, 160 mg.; Choline, 70 gms.; Riboflavin, 3 gms.; Nicotinic Acid, 800 mg.; Folic acid, 45 mg.

to stand until there was a separation in the mixture. The supernatent liquid, a clear dark brown in color, was poured off, and the remaining portion remixed and centrifuged. The supernatent solution was again removed. Extraction of the residue was continued using smaller portions of 66% alcohol until the supernatent solution after centrifuging was practically colorless. A fatty layer formed on top of the liquid after centrifuging. In an effort to remove it, 100 ml. of ether were added to the liquid, but this did not prevent separation. The fay layer was then allowed to go into the liquid portion, and was later removed by pipeting the liquid out from under the fat layer.

The 66% alcohol insoluble portion of the fish solubles was spread out in a porcelain tray to dry on a hot plate. From time to time the pieces were broken up and turned to facilitate drying. After drying, the material was ground to powder in a mill, and was added to the basal ration in lot 5.

The supernatent liquid was filtered with suction to remove any traces of insoluble material. By means of vacuum distillation it was then reduced in volume to that point at which the viscous residue could just be removed from the flask. Since this material still retained a small amount of water, it was dehydrated by the addition of 2000 ml. of absolute alcohol and stirring. This process was repeated with several smaller portions of absolute alcohol. The dehydrated residue was then repeatedly extracted with approximately 250 ml. portions of 95% alcohol until the alcohol extracts became light yellow in color. All the alcohol soluble fractions were combined and reduced in volume by vacuum

distillation. This fraction was designated the 95% alcohol soluble fraction and was added to the ration of lot 6. The 95% alcohol insoluble residue was dissolved in water, in which it was readily soluble, and added to the ration of lot 7.

338 gms. of the 95% alcohol insoluble fraction, representing 10% of the fish solubles, were then dissolved in 500 ml. of water for further fractionation. Basic lead acetate was dissolved in water, boiled, filtered, and added to this fraction in sufficient amounts to precipitate a portion of the proteinaceous materials in the solution. The correct amount was determined by centrifuging small portions, then adding the acetate solution until no further precipitation occurred. The supernatent liquid was then decanted and filtered. The precipitate was repeatedly extracted with hot water, then centrifuged, and the liquid added to the filtrate. Lead was removed as the sulfide, which was then centrifuged and the precipitate thoroughly washed with hot water to extract any absorbed material. Both the lead filtrate and the precipitate were clear brown solutions. The lead filtrate was added to the ration in lot 8, and the lead precipitate in lot 9.

1:20 liver powder was included in the lot 10 ration at a 3% level, and a 95% alcohol insoluble liver fraction representing 4% of the 1:20 liver powder was tested in lot 11.

To study the effect of fish solubles without the B-complex vitamin supplement, fish solubles were added to the basal ration in lot 12.

For comparison purposes a practical chick starter ration, containing animal protein, was included to determine relative growth between this ration and the others, containing principally vegetable protein. (Lot 13) Composition of this ration is given in Table II.

Trial 2

Serving as controls in this study were lot 14, fed the basal ration, lot 15, fed the basal ration supplemented with B-complex vitamins, and lot 16, fed the basal ration supplemented with B-complex vitamins and fish solubles.

A 66% alcohol soluble fraction of fish solubles was prepared by mixing comparable amounts of the 95% alcohol insoluble fraction and the 95% alcohol soluble fraction prepared for trial 1. The resulting fraction was added to the ration in lot 17.

The other fish soluble fractions were prepared in a manner similar to those in the first trial. To 2000 gms. of fish solubles, 580 ml. of water were added, and the two mixed and allowed to stand overnight in an unsuccessful attempt to remove the fat. Then 5300 cc. of 95% alcohol were added, making a 66% alcohol concentration. This solution was treated as previously described in trial 1, centrifuging and re-extracting with 66% alcohol until all the alcohol soluble fraction was removed. This time, however, the volume of the 66% alcohol soluble fraction was not reduced as much as before, the liquid being left in a fairly thin fluid stage. A further effort was made to separate the fat,

TABLE II.

PRACTICAL CHICK STARTER RATION

Ingredient	Per Cent
Ground Kafir	30.0
Wheat Shorts	10.0
Wheat Bran	10.0
Pulverized Barley	10.0
Alfalfa Leaf Meal	10.0
Soybean Oil Meal	10.0
Meat and Bone Scrap	10.0
Dried Buttermilk	8.0
Calcium Carbonate	1.0
Salt	1.0
TOTAL	100.0

The ration was supplemented with Delsterol, 0.025% and Carotene Premix, 0.1%.

first by refrigeration, and then with petroleum ether with little success, so the pipette method was again used.

The lead precipitation was carried out as before, except that the entire fraction soluble in 66% alcohol was used. The lead filtrate was reduced under pressure to a volume of less than two liters.

A mercury precipitation was carried out in the following manner: The lead filtrate was neutralized with acetic acid, as tested with litmus. Mercuric acetate was dissolved in hot water and added to the filtrate, sodium carbonate being added at the same time to maintain the neutrality of the solution. A rich, creamy colored solution was the result. Mercuric acetate was added until there was no further precipitation when portions were centrifuged and the acetate added. Then the solution was again made neutral to litmus. It was stirred using the mechanical stirrer for one-half hour, then was centrifuged to separate the filtrate and the precipitate. The filtrate was cloudy, but further filtering using filter cel proved unsuccessful in removing the cloudiness, whether the solution were basic or acidic or even when more alcohol had been added and it had been refrigerated before filtering.

After adjusting the solutions to a pH between 4 and 5, using litmus and congo red as indicators, the mercury and lead were removed from the mercury filtrate and precipitate and from the lead precipitate as the sulfide. These sulfides were discarded after being removed from the solutions by centrifuging, then washing as previously described. Since the volumes of both the

mercury precipitate and filtrate were so large, they were reduced, using vacuum distillation. Quantities of large white crystals separated, apparently the salts resulting from repeated addition of acids and bases. These were filtered off, washed, and discarded.

The lead precipitate was added to the lot 18 ration. The mercury precipitate, a pale yellow solution, and the mercury filtrate, a clear orange solution, were added to the ration in lots 19 and 20, respectively.

Since the liver preparations in trial 1 had produced optimum chick growth, it was decided to study the effect of decreasing the amount of liver powder in the ration. Therefore, the equivalent of 2% of the liver powder was used in all lots. 1:20 liver powder was used in lot 21, and the 95% alcohol insoluble liver fraction in lot 22. A Norite filtrate and a Norite eluate of the 95% alcohol insoluble liver fraction were used in lots 23 and 24, respectively. The preparation of these fractions was similar to that of fish soluble fractions which will be described in trial 3.

In lot 25 chicks were fed the basal ration plus B vitamin supplements, and were injected with a concentrated solution of APA liver extract at a level of 0.2 cc. per chick per week. These and all other injections were made twice each week, beginning when the chicks were one week old.

Since some authors have reported growth activity in alfalfa leaf meal, and a 90% alcohol insoluble fraction of alfalfa leaf was available in the laboratory, it was incorporated into the

ration of lot 26 at a 10% level.

Trial 3

In this trial as before the first three lots, 27, 28, and 29 were the basal ration, the basal ration supplemented with B vitamins, and the basal ration supplemented with B vitamins and fish solubles.

Fish soluble fractions used were the following: The mercury filtrate fraction prepared for trial 2 was added to the ration in lot 30.

The remainder of the mercury filtrate, 1160 ml., was reduced in volume under pressure to 40 ml. 400 ml. of water were added, and the solution mixed thoroughly. Then 1/4 of this mixture, 110 ml., representing 18% of the original fish solubles, was diluted to about 300 ml. with water, and the pH adjusted to between 4 and 5, using acetic acid, and testing with litmus and congo red indicators. A 10 gm. portion of Norite A was added, and the solution mixed thoroughly, using a glass stirring rod. It was then heated, stirred vigorously, and filtered. This procedure was repeated seven times, until no further color change was apparent. This solution was a pale, clear yellow in color. It will be identified as the Norite filtrate. This fraction was added to the ration of lot 31.

Ammoniacal 70% alcohol was made by adding 2 ml. of concentrated ammonium hydroxide per 100 ml. of 70% alcohol. 200 ml. portions of this alcohol were added to the Norite eluate, using

the above procedure, stirring, heating, and filtering. The Norite was rewashed with the ammoniacal alcohol until the alcohol washings were clear and colorless. This solution, the Norite eluate, was also pale yellow. It was added to the ration of lot 32.

Three other fish soluble fractions were prepared, using alcohol concentrations for extraction not previously tested. To 4000 gms. of fish solubles 1000 ml. of 70% alcohol were added, and mixed thoroughly. The resulting solution was refrigerated overnight. 500 ml. of 95% alcohol were added to the solution, making an alcohol concentration of approximately 75%. After mixing thoroughly and letting the solution stand a short while, the supernatent liquid was decanted. The remaining portion was remixed and refrigerated. The following day the solution was filtered in an attempt to remove crystals which had formed. Since this was unsuccessful, the solution was centrifuged, thus separating the crystals from the 75% alcohol soluble fraction. The crystals were washed in 80% alcohol repeatedly, then centrifuged, separating the crystals and liquid. The liquid was added to the 75% alcohol soluble fraction. Then 500 ml. of 95% alcohol were added to the fraction and mixed, bringing the concentration of the fraction to 80% alcohol. It was then refrigerated overnight.

Since the crystals separated out from the 75% alcohol fractions appeared to be largely made up of inorganic salts, a sample was dried in the oven, then ashed in a muffle furnace. The 28% loss in weight and a positive chloride test using silver nitrate proved the fraction to be largely inorganic matter. Therefore

the fraction was discarded.

After refrigeration, the 80% alcohol soluble and insoluble fractions were separated by centrifuging and filtering. The solution on centrifuging separated into three distinct layers, the soluble fraction, a fatty layer, and the insoluble fraction. The insoluble fraction was washed several times in 85% alcohol, and the washings added to the 80% alcohol soluble fraction. The 80% alcohol insoluble fraction was sucked dry, using a suction filter, and was added to the ration (lot 33). The soluble fraction was set aside for further extraction.

The fat layer was re-extracted twice with 80% alcohol, and the washings added to the 80% alcohol soluble fraction, before the fat was discarded. Sufficient 95% alcohol was then added to this soluble fraction to bring the alcohol concentration from 80% to 85%. It was then treated as previously described, refrigerating, centrifuging, washing, and filtering, to separate the 85% alcohol soluble and insoluble fractions. The 85% alcohol insoluble fraction was included in the ration of lot 34, and the 85% alcohol soluble fraction, of lot 35.

Further studies were made in this trial on the growth stimulating effects of the injected liver concentrate. To determine whether optimum growth could be produced using a lower level of the extract, two lots were injected. In lot 36 the chicks received injections of 0.1 cc. per week, while in lot 37, of 0.04 cc.

Other liver studies were made, using higher levels in this

trial than before. 1:20 liver powder was incorporated at a level of 3% in lot 38, and the Norite eluate of the liver powder was used at 3% in lot 39.

Trial 4

This last trial was run as a re-check on some of the most active fish soluble and liver fractions.

Lot 40 was fed the basal ration, lot 41 the basal ration supplemented with the B-complex vitamins, and lot 42 the basal ration supplemented with the B-complex vitamins and fish solubles. Mercury filtrate was added to the ration in lot 43.

To prepare a Norite eluate of the mercury filtrate of the fish solubles, 110 ml. of the filtrate were diluted to 300 ml., acidified and treated in the manner previously described. After obtaining the Norite eluate, it was divided into two portions, one added to the feed in lot 44, and the other reduced in volume using vacuum distillation to a volume capable of being injected into six baby chicks. The injections were made in lot 45 at a level of 0.25 cc. per week.

The Norite eluate of liver at a 3% level was included in the lot 46 ration. The lot 47 ration was supplemented with APA liver extract injected at a 0.1 ml. level each week.

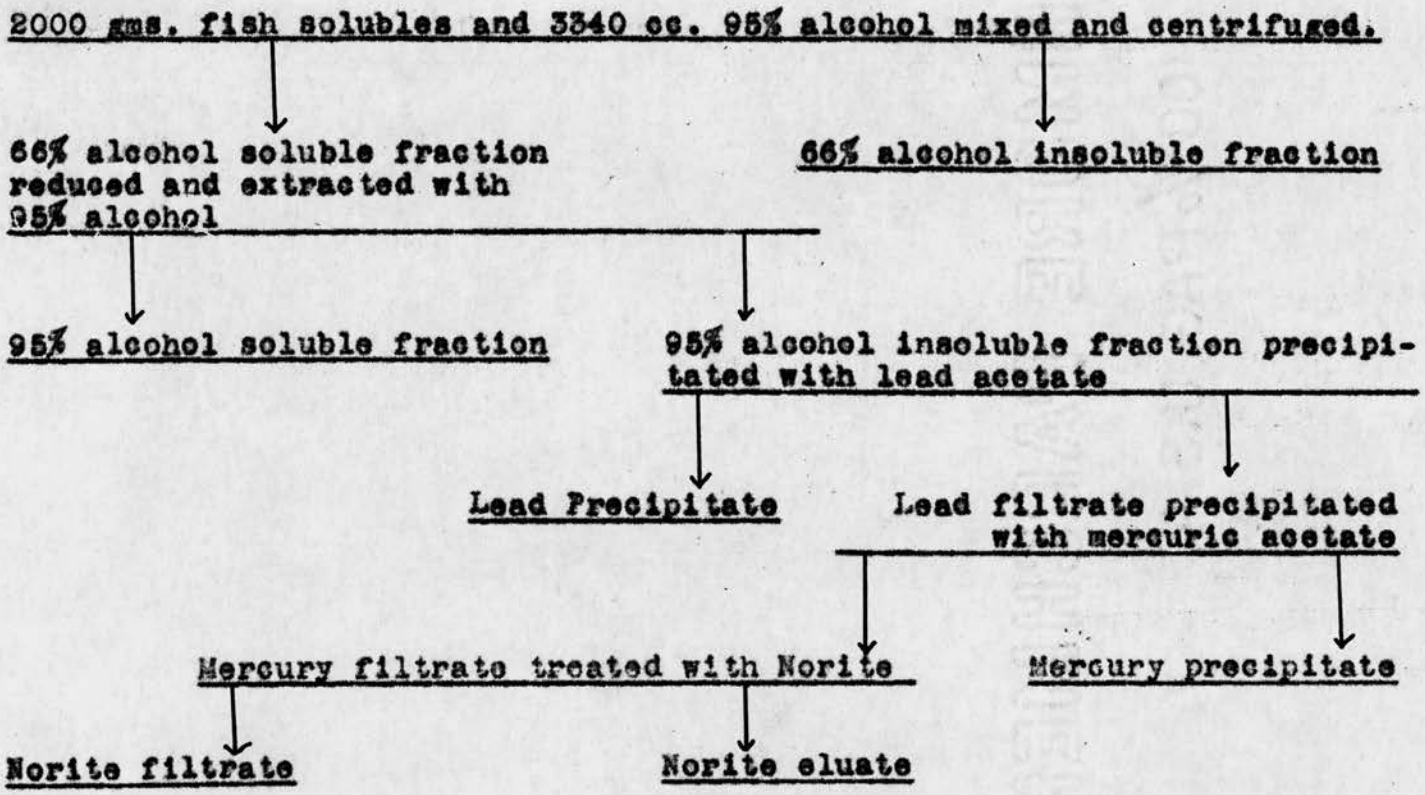
All fractions found active were sampled and the per cent of dry weight and ash determined by oven drying and by ashing in

a muffle furnace at 550° C. Results of these determinations are shown in Table VI.

A summary of the procedures in the fractionation of fish solubles is presented in chart form in Figure 1.

Figure I.

Summary of Chemical Fractionation of Fish Solubles



RESULTS

Results of trial 1, reported in tabular form in Table III and shown graphically in the accompanying growth curves in Figures 2, 3, and 4, indicated that growth promoting activity equal to that of the original fish solubles was found in the 95% alcohol insoluble fraction. The 95% alcohol soluble fraction carried some activity, while almost none was found in the 66% alcohol insoluble fraction. The lead precipitate and filtrate both showed a limited amount of activity, but it was so evenly divided that a lead precipitation method is of doubtful value in concentrating the growth active factor. It was evident from lots 4 and 12 that fish solubles, as such, carried sufficient activity that the B-complex vitamin supplements gave very little boost to the ration.

Apparently liver preparations had approximately the same growth potency as fish solubles, for the 1:20 liver powder and its 95% alcohol insoluble fraction produced gains in weight only slightly less than those obtained using fish solubles.

Extremely low growth of chicks in lot 3 indicated that a supplement of choline alone would not support adequate chick growth, showing clearly the inter-relationship of the B-complex vitamins. The results of Emerson (7) were confirmed in that vegetable protein rations supplemented with fish solubles far surpassed in growth promotion that of the practical chick starter ration containing animal protein.

In trial 2 further efforts at concentrating the growth promoting factor in fish solubles were made, using a mercury precipitation method. Results (Table IV and Figures 5 and 6) showed very

TABLE III.

Trial 1. Average Weight Gains of Chicks on Various Supplements to an All-Vegetable Ration During a 4 Week Period

Lot	Ration	Gain in Weight in Grams per Week				Total Gain in Grams
		Week 1	Week 2	Week 3	Week 4	
1	Basal	22	26	28	34	110
2	Basal, B Vit.	31	51	57	52	191
3	Basal, B Vit., Choline	29	26	31	47	133
4	Basal, B Vit., Fish Solubles	26	61	130	87	304
5	Basal, B Vit., 66% Alcohol Insol.*	27	25	104	75	232
6	Basal, B Vit., 95% Alcohol Sol.*	31	52	96	68	247
7	Basal, B Vit., 95% Alcohol Insol.*	25	72	139	69	305
8	Basal, B Vit., Lead Filtrate*	22	47	59	68	196
9	Basal, B Vit., Lead Precipitate*	24	48	76	70	218
10	Basal, B Vit., 1:20 Liver Powder	20	64	113	87	284
11	Basal, B Vit., 95% Alcohol Insol.**	20	54	95	115	295
12	Basal, Fish Solubles	22	67	78	124	291
13	Practical Chick Starter	19	37	48	79	183

* indicates fractions prepared from fish solubles
** indicates fractions prepared from 1:20 liver powder

Figure II.

Growth rates of chicks in trial 1
on rations 1, 2, 3, 4, and 12.

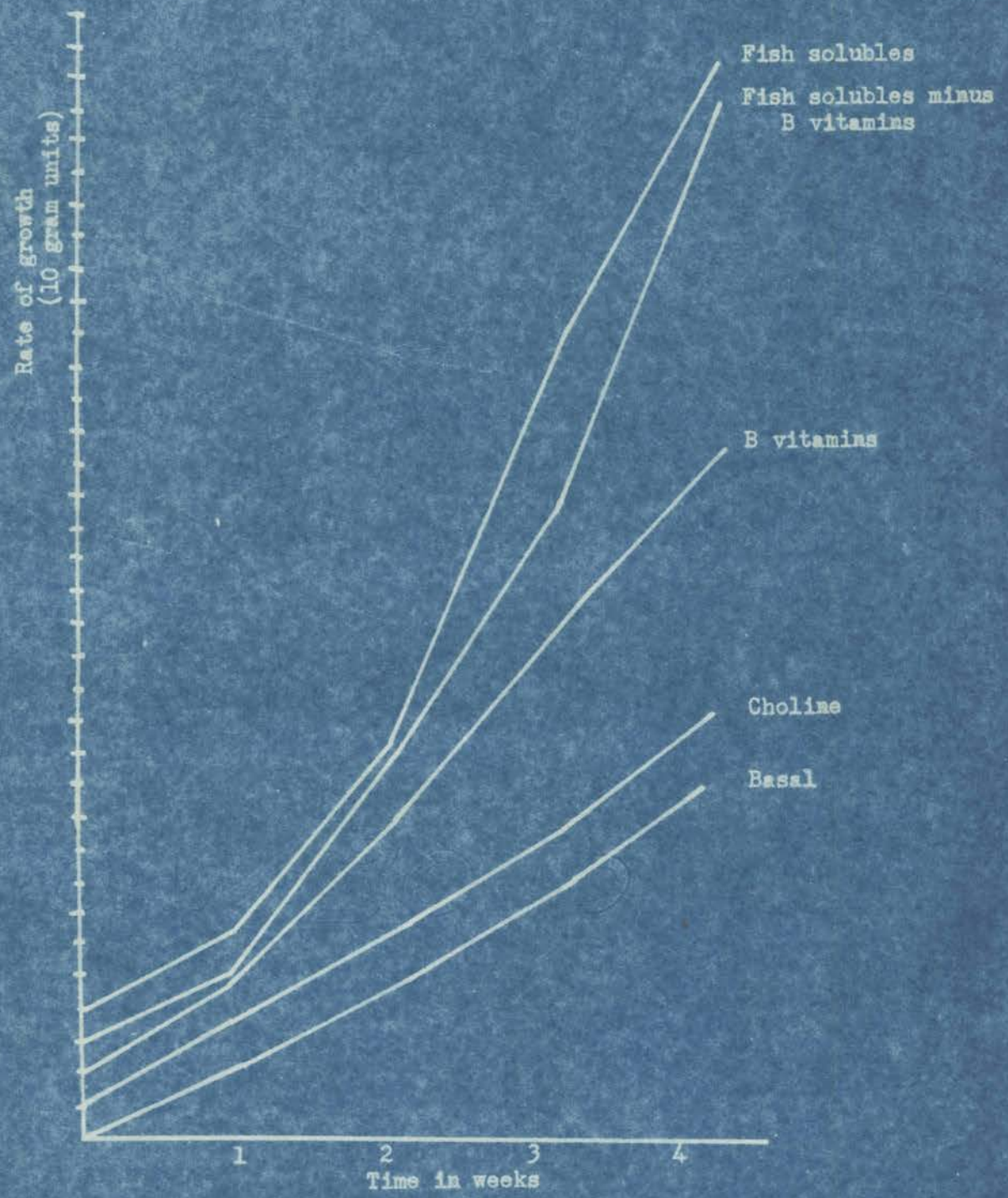


Figure III.

Growth rates of chicks in trial 1
on rations 2, 4, 5, 6, 7, 8, and 9.

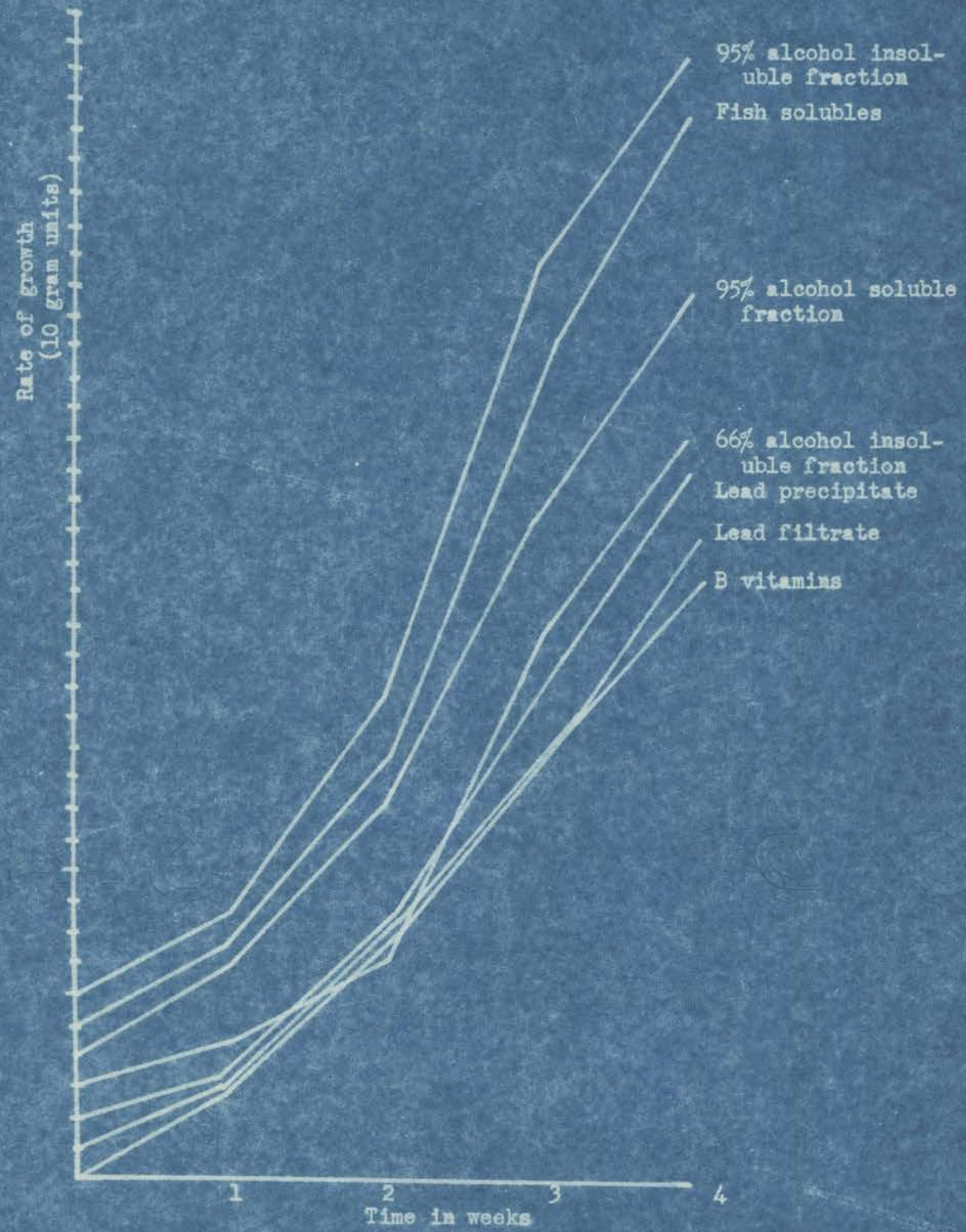


Figure IV.

Growth rates of chicks in trial 1 on rations 2, 4, 10, 11, and 13.

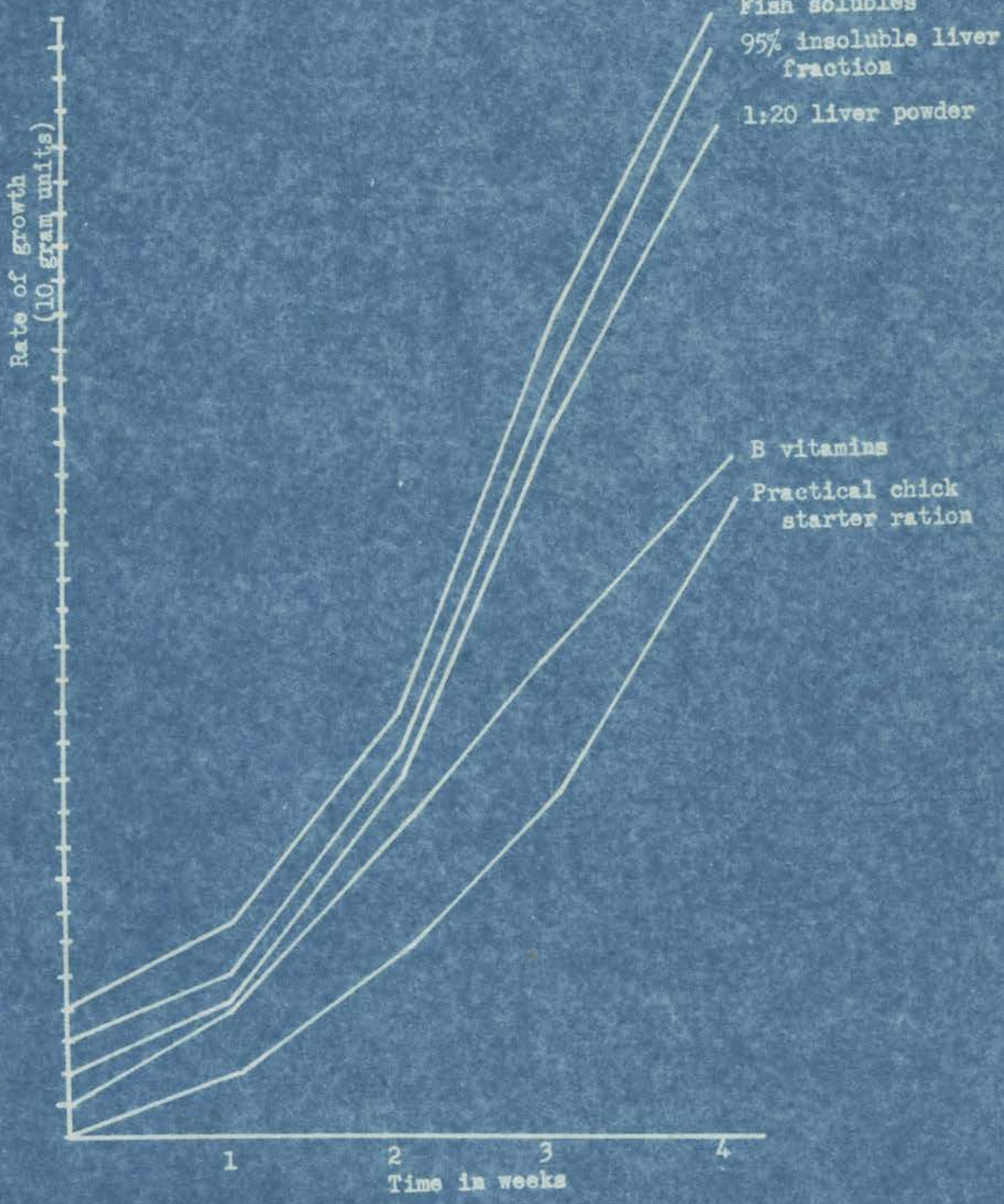


TABLE IV.

Trial 2. Average Weight Gains of Chicks on Various Supplements to an All-Vegetable Ration During a 4 Week Period

Lot	Ration	Gain in Weight in Grams per Week				Total Gain in Grams
		Week 1	Week 2	Week 3	Week 4	
14	Basal	24	46	76	50	191
15	Basal, B vit.	29	56	71	86	242
16	Basal, B vit., Fish Solubles	40	70	104	87	301
17	Basal, B vit., 66% Alcohol Sol.*	33	68	102	111	314
18	Basal, B vit., Lead Precipitate *	33	50	82	97	272
19	Basal, B vit., Mercury Precipitate*	33	55	116	97	201
20	Basal, B vit., Mercury Filtrate *	29	70	92	108	299
21	Basal, B vit., 1:20 Liver Powder	34	66	79	95	274
22	Basal, B vit., 95% Alcohol Insol.**	33	64	86	102	285
23	Basal, B vit., Norite Filtrate **	38	68	74	54	244
24	Basal, B vit., Norite Eluate **	31	61	86	58	236
25	Basal, B vit., Injected APA Extract	37	70	92	110	309
26	Basal, B vit., 90% Alcohol Insol.Alfalfa	24	59	77	75	238

* indicates fractions prepared from fish solubles
** indicates fractions prepared from 1:20 liver powder

Figure V.

Growth rates of chicks in trial 2.
Rations 14, 15, 16, 17, 18, 19, and 20.

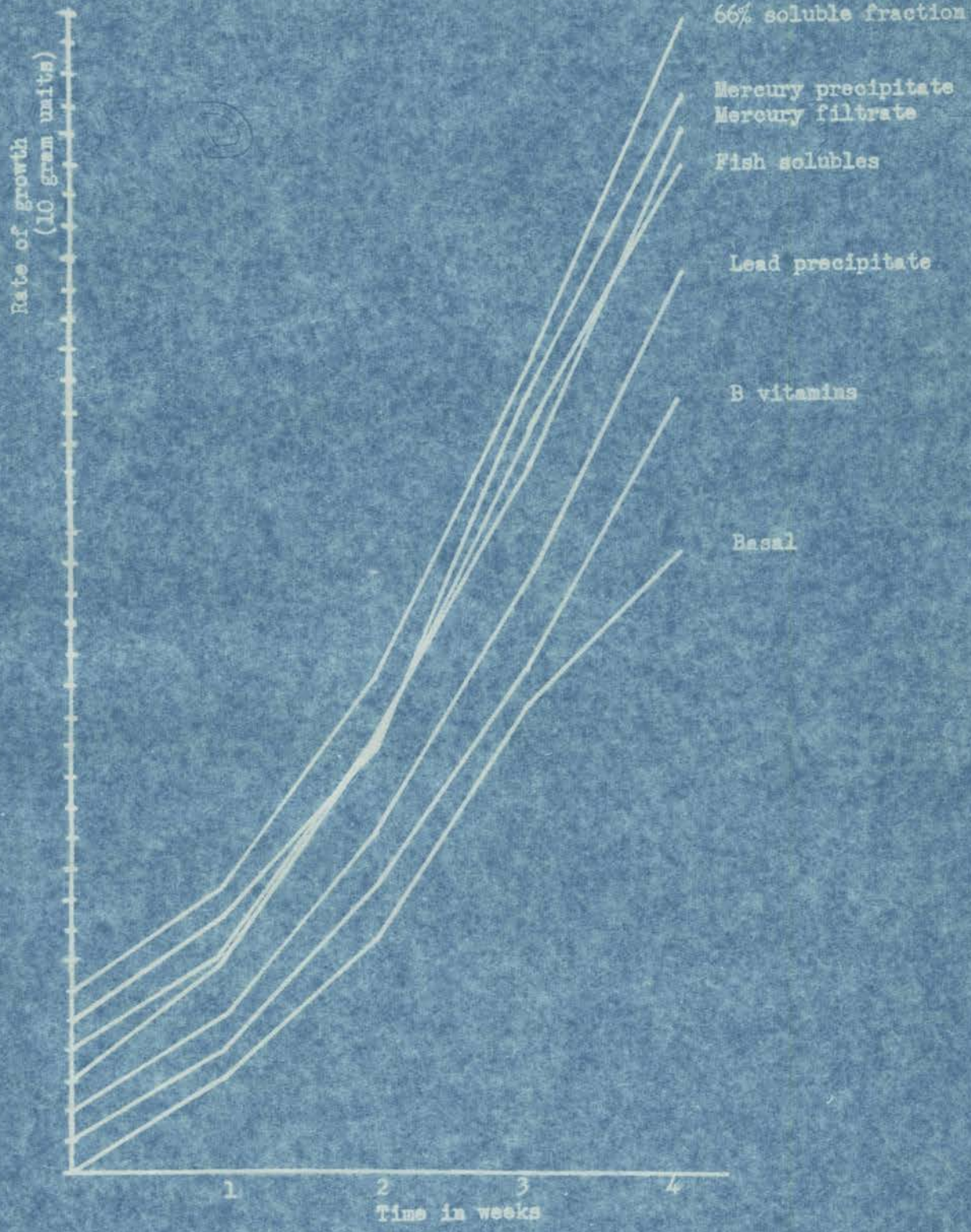
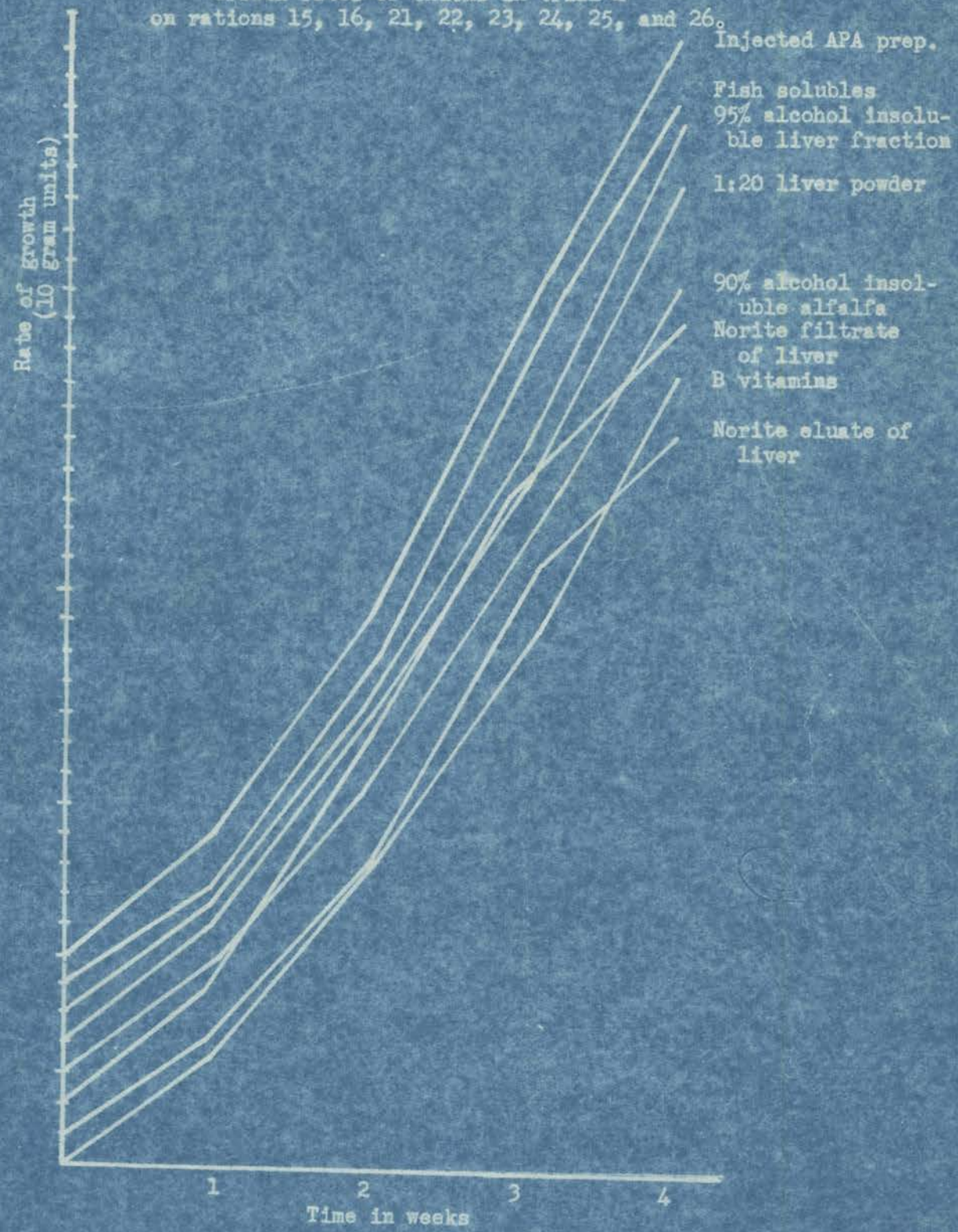


Figure VI.

Growth rates of chicks in trial 2
on rations 15, 16, 21, 22, 23, 24, 25, and 26.



high, but nearly comparable gains in the mercury precipitate and filtrate. These fractions and the 66% alcohol soluble fraction showed activity equal to that of the original fish solubles. Results with the lead precipitate, 1:20 liver powder and the 95% alcohol insoluble liver fraction paralleled those of the first trial.

Extremely high activity was noted with the use of the APA liver injections. The Norite eluate and filtrate of liver, however, as well as the 90% alcohol insoluble alfalfa fraction, showed almost no potency.

Determinations made on the most active fractions for the per cent of dry weight and ash (Table V) showed that a concentration of nearly 60 times that of the original fish solubles had been achieved in the Norite eluate of the mercury filtrate. Results of trial 3 (Table VI and Figures 7 and 8) indicated that this fraction produced growth gains nearly equal to that of the fish solubles. The 85% alcohol soluble fraction and the mercury filtrate showed relatively high activity also, but determinations on these fractions indicated that concentrations of only 2.6 times and 2.5 times, respectively, had been brought about. It was noted that the Norite had been effective in achieving a partial separation of activity. The Norite filtrate of the mercury filtrate, as compared with the Norite eluate, contained very little activity, although a concentration of 10 times was shown by analysis. The 85% alcohol insoluble fraction showed very small comparative gains, and the 80% alcohol insoluble fraction none.

Reducing APA liver injections from 0.2 cc. to 0.1 cc. per week

TABLE V.

DEGREE OF CONCENTRATION OF ACTIVE FISH SOLUBLE FRACTIONS

Fraction	Original Sample Wt. in Grams	Dry Wt. in Grams	Per Cent of Dry Wt.	Ash Wt. in Grams	Per Cent of Ash	Concentration Times
85% Alcohol Soluble	5.416	0.868	16.1	0.074	3.0	2.6
	5.188	0.845	16.3	0.132	3.0	
Mercury Filtrate	5.546	2.037	37.0	0.476	10.0	2.5
	5.940	2.206	37.2	0.512	9.0	
Norite Eluate	4.414	0.089	2.1	0.022	0.5	56.9
	5.483	0.102	2.1	0.026	0.5	
Norite Filtrate	6.703	0.730	11.0	0.268	4.0	10.0
	5.459	0.592	11.0	0.161	5.0	

TABLE VI.

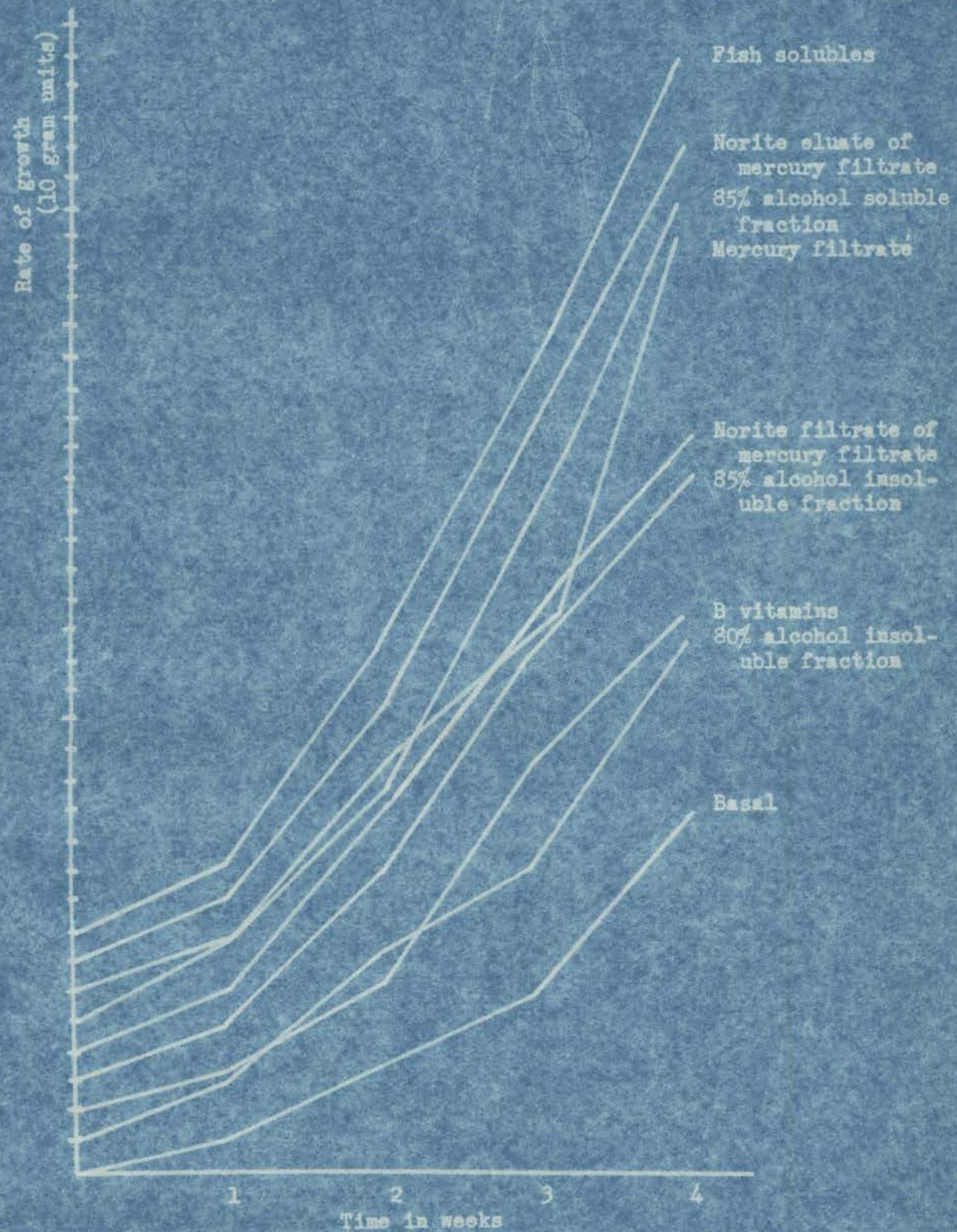
Trial 3. Average Gains in Weight of Chicks on Various Supplements to an All-Vegetable Ration During a 4 Week Period

Lot	Ration	Gain in Weight in Grams per Week Week 1	Week 2	Week 3	Week 4	Total Gain in Grams
27	Basal	18	24	22	55	119
28	Basal, B vit.	18	28	74	47	167
29	Basal, B vit., Fish Solubles	24	71	90	104	289
30	Basal, B vit., Mercury Filtrate*	25	60	45	130	260
31	Basal, B vit., Norite Filtrate*	21	57	63	56	199
32	Basal, B vit., Norite Eluate*	22	61	92	94	269
33	Basal, B vit., 80% Alcohol Insol.*	24	43	29	69	165
34	Basal, B vit., 85% Alcohol Insol.*	21	50	73	53	197
35	Basal, B vit., 85% Alcohol Sol.*	22	47	90	105	264
36	Basal, B vit., Injected APA Extract, 0.1cc.	20	58	88	105	271
37	Basal, B vit., Injected APA Extract 0.04cc.	13	55	67	105	254
38	Basal, B vit., 1:20 Liver Powder	17	61	99	84	261
39	Basal, B vit., Norite Eluate**	13	47	52	171	283

* indicates fractions prepared from fish solubles
** indicates fractions prepared from 1:20 liver powder

Figure VII.

Growth rates of chicks in trial 3
on rations 27, 28, 29, 30, 31, 32, 33, 34, and 35.



resulted in no apparent reduction of growth. Somewhat smaller gains were produced, however, when the injections were cut to 0.04 cc., although they were still comparable to those obtained using 1:20 liver powder. The Norite eluate of liver again showed little activity. This variation from the activity of fish solubles seems somewhat unusual, since other comparable fish soluble and liver fractions have shown similar amounts of activity.

Trial 4, which was run as a re-check on some of the most active fractions of liver and fish solubles, simply confirmed results of previous tests (Table VII and Figures 9 and 10). Injecting the Norite eluate of the mercury filtrate resulted in somewhat smaller gains in weight than when equivalent amounts of the fraction were added to the ration.

TABLE VII.

Trial 4. Average Weight Gains of Chicks on Various Supplements to an All-Vegetable Ration During a 4 Week Period

Lot	Ration	Gain in Weight in Grams per Week				Total Gain in Grams
		Week 1	Week 2	Week 3	Week 4	
40	Basal	12	17	16	45	90
41	Basal, B vit.	13	31	30	51	125
42	Basal, B vit., Fish Solubles	16	52	75	96	247
43	Basal, B vit., Mercury Filtrate*	15	34	58	94	201
44	Basal, B vit., Norite Eluate*	16	34	67	82	199
45	Basal, B vit., Norite Eluate, Injected*	15	43	52	56	166
46	Basal, B vit., Norite Eluate**	17	31	40	71	159
47	Basal, B vit., Injected APA Extract	14	38	75	104	231

* indicates fractions prepared from fish solubles
** indicates fractions prepared from 1:20 liver powder

Figure IX.

Growth rates of chicks in trial 4
on rations 40, 41, 42, 43, 44, and 45.

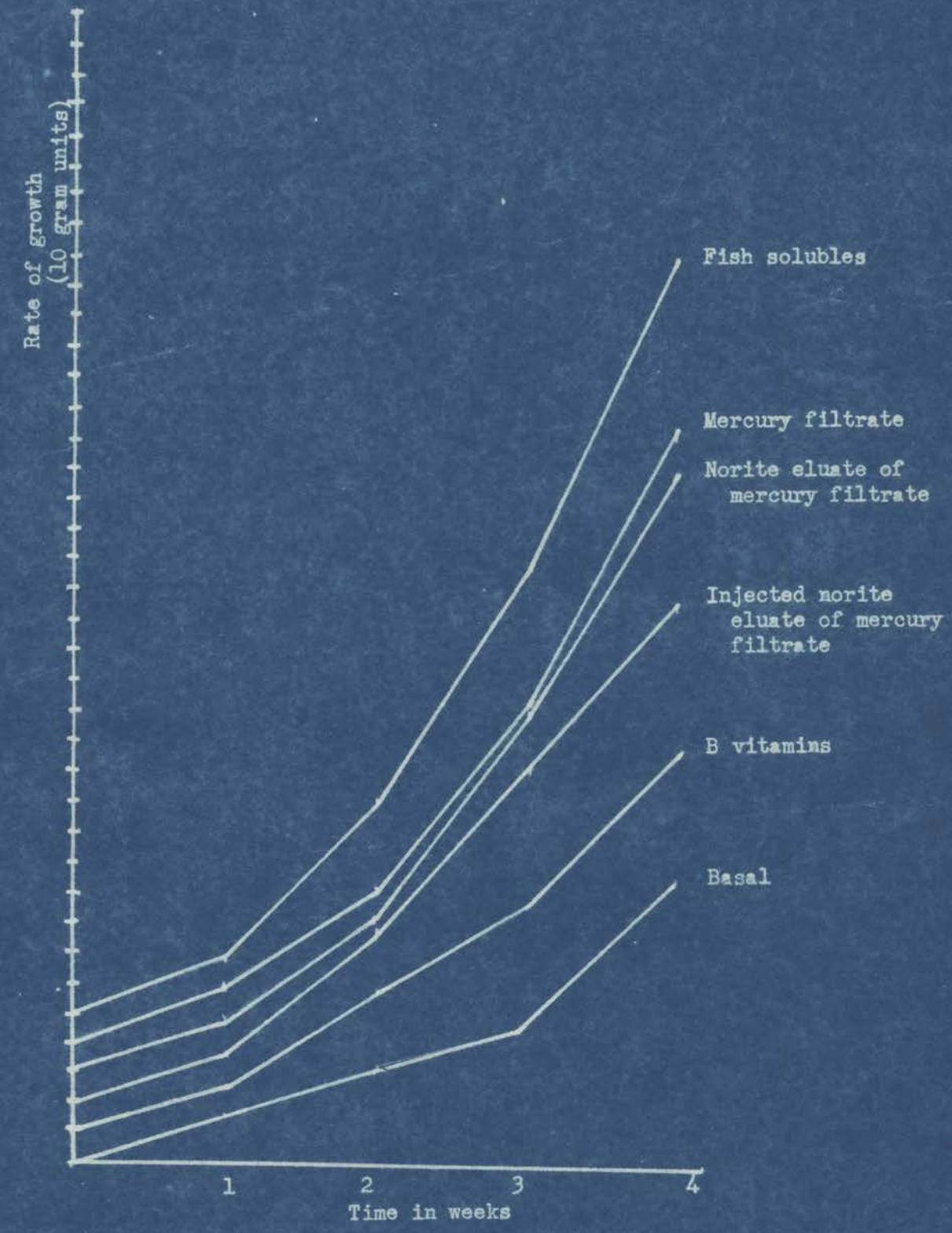
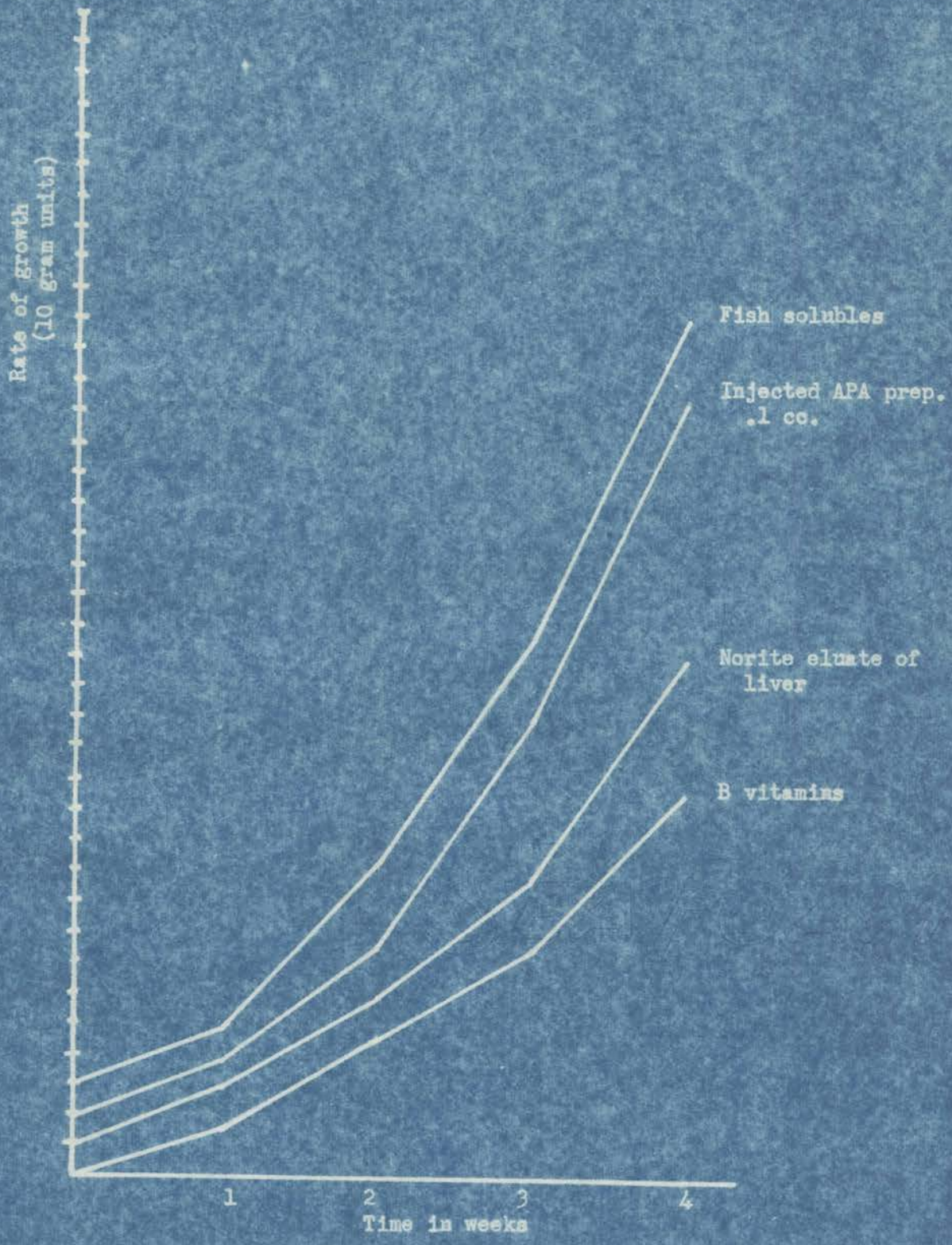


Figure X.

Growth rates of chicks in trial 4
on rations 41, 42, 46, and 47.



CONCLUSIONS

Results obtained from these trials of feeding young chicks an all-vegetable ration supplemented with various fish soluble and liver fractions enable one to draw certain conclusions concerning the chemical and physical properties of the growth promoting factor. In the fish solubles, this growth promoting activity was found in the 66%, 80%, and 85% alcohol fractions. Decreased activity has been consistently found in the 95% alcohol soluble fraction. The decreased solubility of the growth promoting factor in higher alcohol concentrations was also observed during the fractionation of liver.

Addition of basic lead acetate to the 66% alcohol soluble fish soluble fraction produced a lead filtrate containing a fairly high degree of activity. Since a share of the activity was also found in the lead precipitate, it is impossible, without further investigation, to determine whether the lead precipitated some of the active factor, or whether it may have been occluded by the quantities of flocculent matter which was carried down upon the addition of lead. In any event, a large amount of inert material was removed from the lead filtrate by this procedure.

When further precipitation of the lead filtrate was carried out using mercuric acetate, similar results were obtained. Activity was found in the mercury filtrate in fairly high amounts, but was noted to a marked extent also in the mercury precipitate. Once again the action of the mercury upon the active factor is in question. Regardless of whether or not precipitation of the

growth promoting factor was brought about by the mercury however, the resulting mercury filtrate was found to be free of approximately 90% of the inert material found in the original fish solubles. It may be seen therefore that a high percentage of purification had been brought about by these precipitation methods.

Treatment of the mercury filtrate with Norite produced a filtrate containing but little activity and an eluate that was highly active. Thus it is apparent that Norite has an adsorbing effect on the growth promoting factor. However, it is possible that this action is incomplete, since the gains brought about by this Norite eluate were not equal to those of fish solubles, and since the Norite filtrate also contained a small amount of activity. Nevertheless, from a practical standpoint the Norite was the most effective of all methods used, for it produced a highly concentrated active fraction, containing only 1.5% of dry matter, or a 60 fold concentration of the original fish solubles. A higher degree of potency was observed when the Norite eluate was administered orally than when it was injected.

The liver preparations tested were based on a highly active 95% alcohol insoluble fraction made from Wilson's 1:20 liver powder. The fractionations were made in a method similar to those of comparable fish soluble fractions whose preparation have been previously discussed. In general, the growth factor in both fish solubles and liver showed similar properties and effects. However, in the 95% alcohol insoluble liver fraction, the active factor was apparently not adsorbed on Norite to any marked extent, since several trials using the resulting Norite

eluate showed consistently low levels of activity. This variation from the results obtained using a Norite eluate of fish solubles may well be due to a difference in the extraneous materials present in the two solutions, which could easily be responsible for differing reactions, rather than to a difference in the factor itself.

Injection of the anti-pernicious anemia liver extract showed it to be highly effective in promoting growth. Thus it seems extremely likely that the new vitamin B_{12}, isolated from liver and so effective in treatment of Addisonian pernicious anemia, may also be the same highly potent growth factor which has been under observation in these studies.

Growth activity in alfalfa leaf has been reported by some authors, but the results of the lot tested in trial 2, using a dehydrated alfalfa leaf fraction, were completely negative. The 10% level of the leaf equivalent used far exceeded the percentage included in the ration of other sources of the active principle, so these results seem conclusive. They confirm the work of Emerson (7) who found that alfalfa leaf meal added to the ration resulted in no growth stimulus.

SUMMARY

The growth promoting activity of fish solubles and liver preparations for chick growth on all-vegetable rations has been investigated.

Chemical fractionation of the active substance has resulted in partial separation. Results indicate that the greatest degree of concentration of activity was attained in the Norite eluate of the mercury filtrate of the 66% alcohol soluble fraction. The concentration of this fraction was nearly 60 times that of the original fish solubles. Injection of this fraction in the chicks produced growth gains slightly lower than those produced by adding it to the ration.

Liver preparations showed approximately the same growth promoting activity as fish solubles. Of those tested, injection of the APA extract produced the greatest weight gains.

It seems probable that the activity for growth promotion noted in the fish solubles and liver fractions bears some relation to the newly announced vitamin B_{12} isolated from liver, although further research will be necessary in confirming this assumption.

BIBLIOGRAPHY

(1) Berry, E. P., C. W. Carricks, R. E. Roberts, and S. M. Hauge. "Supplementary Effect of Corn Gluten Meal With Soybean Oil Meal." Poultry Science, XXV (January, 1945), 29-33.

(2) Bethke, R. M., H. J. Pensack, D. C. Kennard. "The Influence of the Hen's Diet on Growth of Progeny." Poultry Science, XXVI (March, 1947), 128-131.

(3) Bird, H.R., Max Rubin, and A. C. Groschke. "A Chick Growth Factor in Cow Manure, VII. Its Stability and Solubility." J. Biol. Chem., CLXXIV (June, 1948), 611-619.

(4) Bird, H. R., M. Rubin, D. Whitson, and S. K. Haynes, "Effectiveness of Dietary Supplements in Increasing Hatchability of Eggs and Viability of Progency of Hens Fed a Diet Containing a High Level of Soybean Oil Meal in Chick Rations." Poultry Science, XXV (May, 1946), 285-292.

(5) Brant, A. W. and J. S. Carver. "From One Day of Age Through the First Year on Rations Containing Soybean Oil Meal as a Protein Supplement." Poultry Science, XXVI (November, 1947), 598-603.

(6) Cravens, W. W., W. H. McGinnon, and J. G. Halpin. "The Value of Certain Supplements in Practical Chick Rations Containing Adequate Riboflavin." Poultry Science, XXIV (July, 1945), 305-309.

(7) Emerson, Rebecca W. "Supplementation of All-Vegetable Protein Chick Rations." Thesis, Oklahoma A & M College, 1948.

(8) Grau, C. R. "Fish Solubles for Feeding Poultry." Flour and Feed, XLVI (November, 1946), 46.

(9) Hammond, J. C. "Cow Manure as a Source of Certain Vitamins for Growing Chicks." Poultry Science,XXI (November, 1942), 554-559.

(10) Hammond, J. C. and H. W. Titus. "The Use of Soybean Oil Meal in the Diet of Growing Chicks." Poultry Science, XXIII (January, 1944), 49-57.

(11) Heuser, G. F., L. C. Norris, and J. McGinnis. "Vegetable Protein Concentrates Fed Alone and in Combination With Soybean Oil Meal and Fish Meal as the Chief Supplementary Protein in Chick Starting Rations." Poultry Science, XXV (March, 1946), 130-136.

(12) Johnson, E. L., C. W. Carrick, R. C. Roberts, and S. M. Hauge. "Evidence of a New Growth Factor for Chicks." Poultry Science, XXI (November, 1942), 566-570.

(13) Lassen, S. and E. K. Bacon. "The Use of Condensed Fish Solubles in Poultry Nutrition." Poultry Science, XXV (1946), 486-491.

(14) McGinnis, J. and J. S. Carver. "The Storage of an Unidentified Growth Factor or Factors in the Egg and Its Relation to Chick Growth and Mortality." Poultry Science, XXVI (September, 1947), 457-462.

(15) McGinnis, J., J. M. Stevens, and K. Groves. "Studies on an Unidentified Factor Required for Chick Growth and Livability." Poultry Science, XXVI (September, 1947), 550 (abstract).

(16) Marvel, J. A., C. W. Carrick, R. E. Roberts, and S. M. Hauge. "A Comparison of Soybean Oils and Soybean Oil Meals in Chick Rations Containing Distillers' Dried Solubles." Poultry Science, XXIII (July, 1945), 294-297.

(17) Mishler, D. H., C. W. Carrick, and S. M. Hauge. "Condensed Fish Solubles as a Supplement for Corn and Soybean Oil Meal Chick Rations." Poultry Science, XXVI (September, 1947), 550 (abstract).

(18) Mishler, D. H. C. W. Carrick, R. E. Roberts, and S. M. Hauge. "Synthetic and Natural Vitamin Supplements for Corn and Soybean Oil Meal Chick Rations." Poultry Science, XXV (September, 1946), 479-485.

(19) Nichol, C. A., A. R. Robblee, W. W. Cravens, and C. A. Elvehjem. "Activity of Anti-pernicious Anemia Preparations in Chicks." Jour. Biol. Chem., CLXXI (September, 1947), 419-420.

(20 Novak, A. F. and S. M. Hauge. "Isolation of the Unidentified Growth Factor (Vitamin B_{13}) in Distillers' Dried Solubles." J. Biol. Chem., CLXXIV (June, 1948), 647-651.

(21) Novak, A. F., S. M. Hauge, and C. W. Carrick. "An Unidentified Growth Factor in Distillers' Dried Solubles Essential for the Chick." Poultry Science, XXVI (November, 1947), 604-609.

(22) Patton, A. R., J. P. Marvel, H. G. Petering, and J. Waddel. "The Nutritional Significance of Animal Protein Supplements in the Diet of the Chick." J. Nutrition, XXXI (April, 1946), 485-495.

(23) Robblee, A. R., C. A. Nichol, W. W. Cravens, C. A. Elvehjem, and J. G. Halpin. "Studieis on an Unidentified Growth Factor in Fish Solubles." Poultry Science, XXVI (September, 1947), 553 (abstract).

(24) Rickes, E. L., M. S. Shorb, and R. West. "Crystalline Vitamin B_{12}." Science, CVII (April, 1948), 396-397.

(25) Rubin, M. and H. R. Bird. "A Chick Growth Factor in Cow Manure. I. Its Non-identity With Chick Growth Factors Previously Described." J. Biol. Chem., CLXIII (May, 1946), 393-400.

(26) Rubin, M., H. R. Bird, and I. Rothchild. "A Growth Promoting Factor for Chicks in the Feces of Hens." Poultry Science, XXV (September, 1946), 526-528.

(27) Shorb, M. S. "Activity of Vitamin B_{12} for the Growth of Lactobacillus Lactis." Science, CVII (April, 1948), 397-398.

(28) West, R. "Activity of Vitamin B_{12} in Addisonian Pernicious Anemia." Science, CVII (April, 1948), 398.

(29) Whitson, D., J. C. Hammond, H. W. Titus, and H. R. Bird. "The Use of Soybean Meal in the Diet of Growing Chicks. II. The Effect of Different Grains." Poultry Science, XXIV (September, 1945), 408-416.

(30) Wilgus, H. S. and D. V. Zander. "The Quantity of Animal Protein Required to Supplement Soybean Oil Meal for Growth." Poultry Science, XXIV (January, 1945), 41-45.

(31) Zucker, L. M. and T. F. Zucker. "Zoopherin, A Nutritional Factor of Rats Associated with Animal Protein." Archives of Biochemistry, XVII (January, 1948), 115.

Typist: Jean Huston