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The isolation and the biochemistry of the animal protein factor

John Wyatt West

To the Graduate Council:

I am submitting herewith a thesis written by John Wyatt West entitled "The isolation and the biochemistry of the animal protein factor." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Homer Patrick, Major Professor

We have read this thesis and recommend its acceptance:

Mary V. Reed, Marshall C. Hervey

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 7, 1948

To the Committee on Graduate Study:

I am submitting to you a thesis written by John Wyatt West entitled "The Isolation and the Biochemistry of the Animal Protein Factor." I recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Harry Patrick
Major Professor

We have read this thesis
and recommend its acceptance:

Mary V. Reed
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Accepted for the committee

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Dean of the Graduate School

THE ISOLATION AND THE BIOCHEMISTRY OF THE ANIMAL PROTEIN FACTOR

A THESIS

Submitted to
The Committee on Graduate Study
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

John Wyatt West

June 1948

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INTRODUCTION

There has long prevailed the widespread belief that vegetable proteins, when fed as the sole source of protein in the diet, were deficient in certain essential amino acids. Therefore it was thought that all practical poultry rations should contain some source of animal protein, in order to provide the proper balance of amino acids.

With the advent of the second World War milk by-products, as well as other animal protein concentrates, suddenly became unavailable. Their impending scarcity for use as supplements for vegetable proteins led to the discovery of other sources of the animal protein factor. Cow manure, liver meal, and fish solubles were discovered as sources; linseed meal, cottonseed meal, soybean meal, and peanut meal were found to be deficient in the animal protein factor.

Opinions of some of the leading investigators are at variance concerning the nutritional benefit of animal proteins in their supplementary effects on vegetable proteins. Some feel that the additional benefit is due to an amino acid; others feel that the benefit is due to a vitamin-like substance. The factor present in fish meal will supplement a diet adequate in methionine, but

methionine will not supplement a diet adequate in fish meal. A very small amount of fish soluble concentrates will greatly increase the growth-promoting qualities of a chick ration containing yellow corn meal, soybean oil meal, and adequate minerals and vitamins known to be required.

The main vegetable protein used in the experiments from which these data were obtained was soybean oil meal. Some comparative work was done with cottonseed meal. The method by which a vegetable protein meal is processed has a great influence on the quality of the protein as a feed.

OBJECTIVE

Investigators are generally convinced by now that the animal protein factor is vitamin-like in nature. Experiments which have supplied the data for this report were initiated mainly for the purpose of isolating, purifying, and concentrating the animal protein factor, and to determine by biological means some of the chemical and physical properties of this vitamin-like substance. Fish solubles were used. The fuller's earth eluate was broken down into various fractions, and these fractions were fed to chicks in order to determine the completeness by which each fraction was purified and the biological value of each fraction.

LITERATURE REVIEW

With the outbreak of the second World War the high cost of milk by-products and their impending scarcity for use as supplements for soybean oil meal in poultry rations, it became necessary to look for other sources of animal protein. Berry, Carrick, Roberts, and Hauge (1943) found soybean oil and soybean oil meal to be deficient in available choline. Deficiency of available methionine in soybean oil meal had been previously established. Marvel, Carrick, Roberts, and Hauge (1944) showed that choline chloride and methionine could be used interchangeably in correcting these deficiencies in corn and soybean oil meal chick rations. These authors state that:

> "... if methionine is the deficiency, then it must be assumed that the chick is synthesizing methionine from the added choline chloride when the ration contains a limited amount of methionine. However, if choline is the deficiency, then methionine may act as a precursor of choline for the chick."

Some investigators found that animal proteins were not necessary for good growth in young chicks. Bird and Mattingly (1945) obtained significant growth increases when 0.2 percent dl-methionine was added to the starting and growing mash containing no animal protein. Growth responses due to dl-methionine

slightly exceeded that obtained by supplementation with four percent fish meal. Marvel, Carrick, Roberts, and Hauge (1945) found that the total choline content of broiler rations is not always an insurance that the supply contained is either sufficient or insufficient. Since rapid growth was obtained with the addition of 0.15 percent choline chloride, it was apparent that some of the choline present in soybean crude lecithin was available. Using a ration of yellow corn meal, soybean oil meal, distillers' dried solubles, alfalfa leaf meal, and mineral and vitamin supplements, growth was obtained equal to that of rations containing meat and bone scraps and dried skim milk.

Hammond and Titus (1944) found that soybean meal can be used successfully as the sole protein supplement in growing chick rations. Levels of 35 percent soybean meal, when used alone, were necessary to provide the chicks requirements of cystine and methionine for rapid growth to ten weeks of age. Sardine meal was found to be outstanding as a protein supplement when soybean oil meal is fed at lower levels. Draper and Evans (1944) reported gross values for cottonseed meal, soybean oil meal, herring fish meal, and combinations of these by the procedure of Heiman, Carver, and Cook (1939). The gross values for these concentrates were: cottonseed

meal, 13; soybean oil meal, 66; and herring fish meal, 92 percent. Little supplementation was realized between cottonseed meal and either soybean oil meal or herring fish meal. When cottonseed meal was used as the sole source of protein, the chicks grew very slowly and appeared listless.

Hammond (1942) found that cow manure had a marked beneficial effect on growth in chicks, if added to a diet deficient in riboflavin. Riley and Hammond (1942) observed cow manure to contain androgenic hormones which were manifested by increases in comb and wattle development in chicks without gonad stimulation. Hammond (1944) made a study with dried cow manure. Manure dried at 80°C. or 120°C. was found to be desirable as a substitute for alfalfa leaf meal in turkey rations adequate in Vitamin A and nearly adequate in riboflavin. Temperatures above 80°C. destroyed the androgens. Therefore dried cow manure was found to be a desirable supplement, and a very good source for unrecognized growth factors. In another experiment with chicks, Hammond (1944) found that sardine fish meal did not supplement dried cow manure in producing growth and efficiency of feed utilization.

Whitson, Hammond, Titus, and Bird (1945) found that growth was significantly improved by addition of choline chloride, nicotinic acid, pyridoxin, inositol, and p-amino

benzoic acid to corn and soybean meal rations, in one test, and a combination of choline chloride, nicotinic acid, and bone meal with corn and soybean meal ration in another test. However, these combinations were significantly inferior to rations containing wheat, and to corn rations containing three percent sardine meal or eight percent dried cow manure. Turner (1947) found that pullets on all levels of cow manure outgrew the control groups that received none. Rather than depress sexual maturity the inclusion of five percent and ten percent cow manure was found to hasten the onset of egg production. Rubin and Bird (1947) showed that sun-dried cow manure was as effective as oven-dried manure in stimulating growth and comb development in chicks.

Bird and Mattingly (1945) indicated that the feeding of condensed butyl alcohol solubles derived from yeast fermentation of molasses fed at three percent levels produced a supplementation to rations adequate in riboflavin and other known dietary essentials. This material seemed to be a satisfactory supplement to the ration, due to its content of unrecognized growth factors.

Marvel, Carrick, Roberts, and Hauge (1945), using a simplified ration of yellow corn meal, soybean meal, distillers' dried solubles, alfalfa leaf meal, vitamins and minerals, obtained chick growth equal to that obtained with a practical ration containing meat and

bone scraps and dried skim milk. They concluded that animal protein was not necessary for good growth in young chicks.

Bird, Marsden, and Kellog (1948) found that young turkeys have a critical need for the unknown dietary factor which occurs in cow manure, fish meal, and to a lesser extent in meat meal. In their experiments fish meal was particularly good in meeting this need during early growth; either fish meal or meat meal were effective during later stages.

Berry, Carrick, Roberts, and Hauge (1945) found that two percent fish press water or two percent of fish liver meal would supplement a corn and soybean meal ration containing adequate minerals and vitamins. By fractionation they found the active factor to be present in the alcohol-soluble fraction, and not in the alcohol precipitate which contained most of the protein.

Cravens, McGibbon, and Halpin (1945) found condensed fish press water or ground fish viscera to be effective in supplementing diets adequate in riboflavin. A combination of dried skimmilk and fish press water proved to be the most satisfactory combination in their series of tests.

Lassen and Bacon (1946) obtained good results with condensed fish solubles in poultry rations, and found that

levels as high as twelve to thirteen percent of a ration can well be tolerated and produce proportional increase in growth. Above these levels, its high mineral content in some way counteracts its growth promoting qualities. They found that condensed fish solubles can replace skimmilk powder, pound for pound, in a growing mash.

Patton, Marvel, Petering, and Waddell (1946) discovered that the factor present in fish meal would support growth to a greater extent than would methionine, and would supplement a ration adequate in methionine; but methionine would not supplement a ration containing fish meal. Their conclusion stated that the growth promoting activity was due to unrecognized factors.

Bird, Rubin, and Groschke (1947) reported that a certain amount of the growth stimulating factor could be transmitted from parent to offspring, thus stimulating early growth in the chick.

Patrick and Stephenson (1947) concluded that one percent fish solubles is equal to fish meal as a carrier of unidentified growth promoting factors. The factor or factors involved, contained in the alcohol filtrate of fish solubles, are water and alcohol soluble, stable in acid or alkaline medium, and are not destroyed by relatively high temperature. They are dialyzable, readily adsorbed on fuller's earth, readily eluted with

hot, absolute methyl alcohol, and appear to be vitamin-like in nature. It was found that a saturated solution of ammonium sulfate will partially precipitate the factor.

German, Schweigert, Pearson, and Sherwood (1948) made a preliminary study on the value of condensed fish solubles for turkey poults. Data based on gains in live weight indicate that fish solubles is a valuable supplement for poults fed a milo maize and soybean oil meal ration adequate in riboflavin.

Rubin and Bird (1946a,b,) reported that an all-plant protein ration containing 35 percent of commercially heated soybean oil meal produced suboptimal growth of chickens unless supplemented with fish meal, cow manure, or extracts of cow manure. Evidence presented by Rubin and Bird (1946a) showed that the growth factor of cow manure is not identical to the Lactobacillus casei factors (from liver, yeast, or fermentation residues), factors U, R, or S, vitamins B_{10} or B_{11}, synthetic folic acid, or pyracin lactone. Rubin and Bird (1946b) found that the growth factor they extracted and concentrated from cow manure stimulates chick growth on a diet free of animal protein, optimal feeding levels being 3.75 to 7.5 mg. per 100 gm. of ration. They found the factor to be transmissable from hen to chick.

Rubin and Bird (1947) found that the feeding of 70 percent soybean oil meal to young chickens inhibited growth and increased mortality. Addition of the growth factor of cow manure counteracted both of these effects, but the addition of methionine failed to do so. The growth factor of cow manure improved the nutritional value of a chick diet containing raw soybean oil meal as the only protein concentrate, but not to the extent that it improved a diet containing heated soybean oil meal.

PROCEDURE

Experimental Animals

New Hampshire chicks, obtained from the Nichols Poultry Farm, New Market, Tennessee, were used in this study.

Quarters and Care of Animals

Battery brooders, equipped with thermostatically-controlled heating units and hardware cloth floors, were used for brooding the chicks. These brooders were housed in a steam-heated building with an uncontrolled ventilation system. Day-old chicks were kept at a temperature of approximately 35°C., the temperature then being gradually lowered to a room temperature of approximately 25°C.

Upon arrival from the hatchery the chicks were fed a depleting diet of yellow corn meal and water for three or four days. By random selection the chicks were then placed on their respective test rations. Feed and water were kept before them at all times.

During the test the chicks were observed daily, water troughs and dropping pans being cleaned regularly to maintain sanitary conditions. At the end of five weeks they were weighed and the weights recorded. Upon

TABLE I

BASAL RATION

Ingredient	Percent
Yellow corn meal . . . . . Enough to total	100.00
Vegetable protein[a] . . . . To constitute 12.00% Protein	
Alfalfa leaf meal	2.25
Steamed bone meal	2.00
Salt	0.75
Vitamin A (6000 I. U.)	0.04
Vitamin D_3 (2000 A. O. A. C.)	0.03
Manganese Sulfate	0.03
Riboflavin	0.0005

[a] Expeller soybean oil meal, solvent cottonseed oil meal, and hydraulic cottonseed oil meal were used as the vegetable protein supplements.

completion of each experiment, the battery brooders were thoroughly cleaned and the removable parts steamed before use with a new group of chicks.

Rations

The basal ration, as shown in Table I, was composed mainly of yellow corn meal and soybean oil meal, with mineral and vitamin supplements in sufficient amounts to meet the known requirements. Supplementation studies were made with methanol solubles and fuller's earth eluate, which were prepared in our laboratory from the methanol soluble portion of fish solubles.

Preparation of the Fractions from Fish Solubles

Fish solubles were supplied by Van Camp Sea Food Company, Inc. of Terminal Island, California. This brown syrupy liquid included the body fluids of fish and the parts of tissue which had been partially digested by autolytic enzyme activity. It contained approximately fifty per cent dry matter, about twenty-seven per cent protein and seven per cent fat.

The methanol solubles were prepared by adding approximately nine parts of methanol to one part of fish solubles. Most of the protein was precipitated by the methanol. The methanol precipitate was washed three to four times with methanol. Filtrate portions

were removed each time by decantation, combined, distilled in vacuo to a concentration of about fifty per cent dry matter, and stored at low temperature for future use.

Fuller's earth eluate was prepared as follows; the concentrated filtrate portion of methanol solubles was diluted with water until an aqueous solution of approximately ten per cent dry matter was obtained. Then the pH was adjusted to one by adding dilute hydrochloric acid solution. Fuller's earth adsorbent was added at the rate of about one gram per gram of dry matter of the filtrate, thoroughly stirred for thirty minutes, and allowed to settle. The straw-colored filtrate portion was removed by decantation and discarded. The fuller's earth absorbate was over-dried at low temperature and then eluted three times with hot methanol, the eluate being removed each time by decantation. Excess alcohol was removed from the combined eluates by distillation in vacuo, until a dry matter of approximately thirty per cent was obtained.

One 200-gram portion of the eluate was placed in a cellophane membrane, sealed and suspended vertically with the lower end of the membrane in a tall half-liter cylinder of hot water. A pressure developed within the membrane, the level of the liquid rising approximately ten centimeters. The liquid outside the membrane developed a yellow color, indicating that some of the

eluate was diffusable. After five additions of hot water, the diffusable and non-diffusable fractions were stored separately for subsequent use in supplementation studies.

A second 200-gram portion of the eluate was diluted with approximately one liter of water and the pH adjusted to one with dilute hydrochloric acid solution. Norite-A, decolorizing carbon, was added at the rate of one gram per gram of dry matter of the eluate, and adsorbed by thorough mixing for thirty minutes. Four subsequent adsorptions were made of the decanted filtrate fraction. Then the filtrate and adsorbate fractions were stored at low temperature for future use.

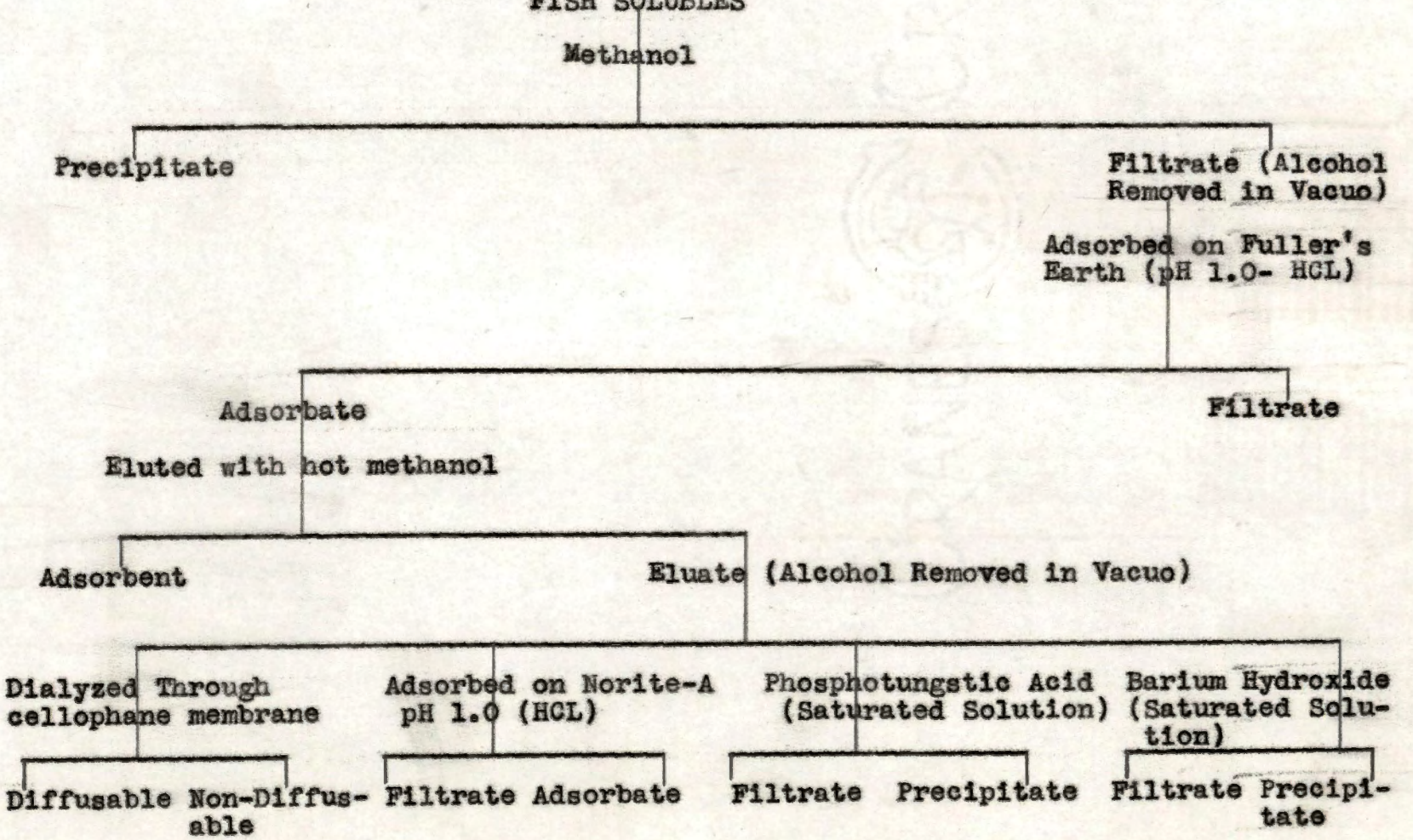
To a third 200-gram portion of the eluate was added an equal volume of saturated barium hydroxide solution. A white precipitate formed, presumably a barium proteinate. The filtrate fraction was removed by decantation. Dilute sulfuric acid solution was added to remove the barium from the filtrate as insoluble barium sulfate. Then the barium proteinate was decomposed with dilute sulfuric acid solution to precipitate the barium as insoluble barium sulfate. Both the filtrate and precipitate fractions were stored for feeding investigations.

The fourth 200-gram portion of fuller's earth eluate was treated with an approximately equal volume of saturated phosphotungstic acid solution. A white precipitate formed, presumably a protein phosphotungstate. The filtrate fraction was removed by decantation. To both the filtrate fraction and an aqueous suspension of the precipitate fraction was added an excess of saturated barium hydroxide solution to remove the heavy phosphotungstic ions as barium phosphotungstate. To the decanted filtrate portions of both these fractions was added dilute sulfuric acid solution to remove the excess barium as insoluble barium sulfate. Then both the filtrate and precipitate fractions of the original eluate were separately stored for future use in supplementation studies.

A summary of the entire fractionation procedure is included in Table II of this report.

TABLE II

SCHEMATIC OF FISH SOLUBLE FRACTIONATIONS



RESULTS AND DISCUSSION

Experiment I

The primary purpose of this experiment was to study and compare the supplementary effects of the methanol-soluble fraction of fish solubles on vegetable proteins known to be deficient in certain nutrients found in animal protein concentrates. Secondarily, the experiment would afford some basis for comparison of the biological value of vegetable protein supplements in terms of chick growth and livability. Expeller-type soybean oil meal and both solvent and hydraulic cottonseed oil meals were used.

Much has been said about toxic substances present in cottonseed oil meals and the undesirability of their use in chick rations. Results as shown in Table III of this report show that growth obtained with good cottonseed oil meal was superior to that obtained with expeller soybean oil meal. No toxic effects were observed in this experiment. The biological value of cottonseed oil meal is greatly influenced by the method of manufacture; the same holds true in the case of soybean oil meal. Recent indications are that properly-processed cottonseed oil meals show considerable promise for use as protein supplements in chick rations.

All groups receiving the methanol-soluble fraction of fish solubles made faster and greater gains than those receiving only vegetable protein. Comparing Groups 1 and 4, the average weight of Group 4 receiving methanol solubles was almost double that of the negative control. Relatively speaking, expeller soybean oil meal is a poorer protein supplement than either of the cottonseed oil meals; this is a possible explanation as to why methanol solubles produced such great supplementary effects in the case of the expeller soybean oil meal.

Contrary to previous results reported from our laboratory, a new shipment of cottonseed oil meals proved to be superior to expeller-type soybean oil meal. Futhermore, this shipment of hydraulic cottonseed oil meal was found to be superior to the solvent-extracted cottonseed oil meal.

Average weights for Groups 3 and 6 when compared to Groups 2 and 5, respectively, indicate that hydraulic cottonseed oil meal is superior to solvent-extracted cottonseed oil meal when fed alone or when supplemented with the animal protein factor. Addition of four percent corn gluten meal to the negative control ration fed to Group I produced only a small amount of supplementation, as evidenced by the average weights recorded for Group 7.

TABLE III

SUPPLEMENTARY VALUE OF THE METHANOL SOLUBLE FRACTION OF FISH SOLUBLES ON VEGETABLE PROTEIN RATIONS

Group	Supplement To Basal	Weight 5 Weeks
		Grams
1	Expeller Soybean Oil Meal Basal	152
2	Solvent Cottonseed Oil Meal Basal	195
3	Hydraulic Cottonseed Oil Meal Basal	211
4	Exp. S.O.M. Basal + Methanol Solubles[a]	295
5	Solvent C.S.O.M. Basal + Methanol Solubles[a]	285
6	Hydraulic C.S.O.M. Basal + Methanol Solubles[a]	328
7	Exp. S.O.M. Basal + Corn Gluten Meal[b]	177
8	Exp. S.O.M. Basal + Corn Gluten Feed[c]	154

[a] Fed equivalent to 4.0 percent Fish Solubles.

[b] Added at 4.0 percent level.

[c] Added at 6.0 percent level.

Results in Group 8 represent a negligible amount of growth-promoting activity due to the addition of six percent corn gluten feed.

In this experiment there was no appreciable difference in degree of livability between the eight groups to five weeks of life. At the beginning of the test, 100 chicks were randomized into eight groups; approximately ninety percent livability was obtained in all groups.

A duplicate of this experiment was later run in which solvent soybean oil was used in the place of expeller-type. Similiar results were obtained, except solvent soybean oil meal produced growth superior to either solvent or hydraulic cottonseed oil meal.

Experiment II

This experiment was planned for the purpose of assaying biologically the relative potencies of methanol solubles, fuller's earth eluate, and two samples of a liver extract concentrate. Group I was again used as the negative control and levels one, two, and three percent fish meal in Groups 2,3, and 4, respectively, provided the standard growth curve by which comparisons were made. These results are shown in Table IV.

TABLE IV

SUPPLEMENTARY VALUE OF VARIOUS ANIMAL PROTEIN FACTOR CONCENTRATES IN A RATION CONTAINING SOYBEAN OIL MEAL

Group	Supplement to Exp. S.O.M. Basal	Weight 5 Weeks
		Grams
1	None	242
2	1.0 percent fish meal	325
3	2.0 percent fish meal	378
4	3.0 percent fish meal	402
5	J-495 (1 ml. per Kgm.)[a]	380
6	J-500 (2.5 ml. per Kgm.)[a]	376
7	0.10 percent fuller's earth eluate	276
8	0.20 percent fuller's earth eluate	328
9	0.50 percent methanol solubles	326

[a] J-495 and J-500 were supplied by Dr. T. H. Jukes of Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. These concentrates were prepared from liver extracts.

Samples J-495 and J-500 which were fed to Groups 5 and 6, respectively, produced growth increases approximately equal to that obtained in Group 3 which received two percent fish meal. Average weights recorded for Groups 7 and 8 receiving 0.10 and 0.20 percent fuller's earth eluate, respectively, indicate supplementary growth effects equivalent to one percent fish meal at the higher level and growth effects approximately equal to 0.50 percent fish meal at the lower level. The ration fed to Group 9 containing 0.50 percent methanol solubles produced average weight increases equivalent to one percent fish meal and 0.20 percent fuller's earth eluate. On the basis of these data the fuller's earth eluate is a much more potent concentrate of the animal protein factor than is the methanol-soluble fraction of fish solubles.

Lederle samples J-495 and J-500 are concentrates prepared from liver extracts which are used in the treatment of pernicious anemia. Since concentrates of both the animal protein factor and the anti-pernicious anemia factor exhibit the common characteristic of growth-stimulating activity in chick rations, it seems reasonable to assume that the two factors are somewhat related.

Experiment III

Having determined that the fuller's earth eluate retains the greater part of the activity found to be

TABLE V

SUPPLEMENTARY VALUE OF FRACTIONS OF FULLER'S EARTH ELUATE IN A RATION CONTAINING SOYBEAN OIL MEAL

Group	Supplement to Basal	Weight 5 Weeks
		Grams
1	None	236
2	Fuller's earth eluate	292
3	Non-diffusable	248
4	Diffusable	342
5	Norite filtrate	281
6	Norite adsorbate	269
7	Ba $(OH)_2$ filtrate	283
8	Ba $(OH)_2$ precipitate	218
9	Phosphotungstic Acid filtrate	270
10	Phosphotungstic Acid precipitate	230

present in fish solubles, attempts were made to further concentrate and purify the animal protein factor. The results are shown in Table V.

Group I was again used as the negative control, their ration containing soybean oil meal as the only protein supplement. Group 2 received fuller's earth eluate and was used as the positive control.

Some workers have reported difficulty in dialyzing the growth promoting factor. The chicks in Group 3, which received the fraction of eluate that did not diffuse through the cellophane membrane, attained growth only slightly greater than the negative control. In Group 4 the chicks were fed the fraction of eluate that diffused or passed through the cellophane membrane, and their growth response was much greater than that of the positive control. During the dialysis procedure a great amount of pressure developed within the membrane. These data would indicate that the growth factor is dialyzable, and will diffuse through a cellophane membrane when subjected to pressure from within a tightly closed volume.

Reports have been made by other investigators that the growth promoting factor is not adsorbable on norite. For this experiment Norite-A, decolorizing carbon, was used. Chicks in Group 6 receiving the norite adsorbate attained growth superior to the negative control group, but inferior to the positive controls and slightly

inferior to Group 5 receiving the norite filtrate fraction of fuller's earth eluate. However, in the duplicate of this experiment, a much greater growth response was obtained with the group receiving the norite adsorbate fraction of eluate. These data clearly indicate the factor to be partly adsorbable on Norite-A at strongly acid pH levels. Since a part of the activity is retained in the norite filtrate fraction of the eluate, previous reports from this laboratory stating that the factor exists in two forms are hereby confirmed.

The chicks in Groups 7 and 8 were fed basal ration supplemented with the barium hydroxide filtrate and precipitate fractions of eluate, respectively. Since the growth obtained with the filtrate fraction was almost equal to that of the positive control group, and the group receiving the precipitate fraction attained growth comparable or less than the negative control group, these data indicate that the factor is not precipitable as the barium salt. During the preparation of the two fractions, much of the color and impurities were removed in the procedure. Thus the barium filtrate fraction of the eluate retained most of the growth factor, and fractionation procedures eliminated many of the impurities.

Another portion of the fuller's earth eluate was precipitated with a saturated phosphotungstic acid solution. Group 10 which was fed the precipitate fraction showed an average gain slightly less than the negative control group. Group 9, which received the filtrate fraction after precipitation, showed considerably more gain than the negative controls, but somewhat less than the positive control group. This indicates that the growth factor is not precipitable with saturated phosphotungstic acid solution.

CONCLUSIONS

From the growth results obtained in the foregoing experiments, it seems reasonable to assume the following conclusions:

1. Fish solubles is a rich source of the animal protein factor.

2. The growth-promoting factor or factors are contained in the methanol filtrate fraction of fish solubles, and are both water and alcohol soluble.

3. The animal protein factor is readily adsorbed on fuller's earth at pH one, and readily eluted with hot absolute methanol.

4. Fuller's earth eluate retains a very high percentage of the growth-promoting activity in fish solubles, and is in a more purified state.

5. These factors are stable at relatively high acid and alkaline pH levels.

6. When subjected to considerable pressure in a closed membrane, the growth factor is dialyzable through a cellophane membrane.

7. At strongly acid pH levels, the factor or factors are adsorbable on Norite-A.

8. Addition of saturated solutions of barium hydroxide and phosphotungstic acid will not quantitatively precipitate the factor from fuller's earth eluate.

9. The greater portion of the growth factor is retained in the filtrate portions of the eluate after precipitation with saturated barium hydroxide and phosphotungstic acid solutions.

10. The animal protein factor may be somewhat related to the anti-pernicious anemia factor found in liver extracts.

11. Because of the relatively small amounts needed to produce pronounced supplementation in terms of growth, the animal protein factor appears to be vitamin-like in nature.

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