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The Adequacy of Synthetic Rations for the Growth of Chicks

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The Adequacy of Synthetic Rations for the Growth of Chicks

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The attention of nutritional physiologists has been directed for many years to the identification of the nutrients that are required for the animal body. Considerable study has been devoted to the various quantitative adjustments, but the problem of qualitative demands is still one of major interest.

HISTORICAL

Several excellent summaries[1]* of the literature have been published by other workers in this field thus making extended comment unnecessary. We shall therefore note especially studies conducted with baby chicks that were fed synthetic diets, and so strictly comparable to ours.

The earlier attempts at studying the nutritional requirements of chickens were uniformly unsuccessful. Such studies meant that the birds must be reared in confinement, and the first investigators failed to accomplish this satisfactorily with any ration tried. Apparently Osborne and Mendel[2] were the first to attain any real degree of success in this type of investigation. Their chicks suffered from leg weakness but made gains in weight, one weighing 1278 and another 1267 grams at the end of 178 days. These authors attributed their success, in large measure, to the use of a considerable quantity of roughage.

Hart, Halpin, and Steenbock[3] confirmed the statements of Osborne and Mendel that chicks require indigestible ballast in their rations. In their most successful feeding trial the ration consisted of casein 18, dextrin 37, butter fat 15, dried yeast 15, salts 5, and paper 10. All of the chicks were weighed and the weights varied from 480 to 710 grams at the end of 15 weeks. The rate of growth was subnormal, and the best specimens obtained had distinctly ruffled feathers at 9 to 13 weeks. A chicken photographed at 16 weeks was apparently normal in appearance.

Sugiura and Benedict[4] have recently stated that the pigeon does not require vitamin A, and have drawn the general conclusion that this vitamin is dispensable in avian nutrition. Hoet also published data indicating that vitamin A is not required by the pigeon. As a result of his observations[5] over a longer feeding period however he reversed his position, and stated that though the requirement for this vitamin is low, still vitamin A is not dispensable.

*This refers to corresponding number in bibliography.

Emmett and Peacock[6] established beyond question that chicks do require vitamin A. They obtained practically normal growth on a synthetic ration, and if any of the chicks were abnormal in any respects the fact was not noted. There is some doubt in our minds as to whether their ration would have been equally successful if begun with baby chicks.

A considerable advance in avian nutrition was made when Hart, Halpin, and Steenbock[7] announced that leg weakness can be prevented by the administration of cod liver oil, and that its symptoms are in some respects characteristic of rickets.

Not long afterwards Hughes[8] in a popular article described observations which led him to believe that leg weakness of chicks is in reality rickets, and that it is prevented by exposure to direct sunlight. The observations of Hughes have been abundantly confirmed by Hart, Steenbock, Lepkovsky, and Halpin.[9] In their most recent paper, the Wisconsin Group[10] reopened the question of the chicks' requirement for vitamin A, and again all evidence indicated that they cannot dispense with this vitamin. In some of their feeding trials synthetic diets were used, with a considerable degree of success. Even in the most favorable cases however growth was subnormal. The ration consisted of casein 18, salts 5, agar 2, yeast 15, dextrin 60, plus the unsaponified residue of 5 parts of cod liver oil.

It is clearly established we believe that vitamin C is not required by the chick.[11] At present then it is generally agreed that in addition to a proper adjustment of the supply of protein, salts, and energy, chickens require three vitamins, A, B, and D (antirachitic), unless the birds are exposed to ultra violet rays. In this case the antirachitic vitamin is dispensable.

EXPERIMENTAL

The object of this investigation is to determine the adequacy of synthetic rations for the growth of chicks. This means that the chickens must be grown in confinement, and that rations of known composition must be fed. It also involves special precautions to prevent the birds from receiving any food, insects and etc., other than that provided for experimental use.

Experimental Chicks.—We have consistently used single-comb White Leghorns in our investigations. In our earlier experiments, chicks were obtained from the Poultry Department of the University of Missouri. Later they were purchased from a local hatchery. The chicks were taken from the incubator on the day of hatching, weighed, and placed in the experiment immediately.

Quarters.—After weighing, the chicks were transferred to brooders, located in a substantial stone building. The room is fairly well lighted by two east windows, but direct rays of the sun do not reach the chicks. The quarters are satisfactory, except in cold weather the temperature may be lower than desirable. The building is heated with steam and the brooders themselves are heated with electric hot plates. When the chicks are 6 to 8 weeks old, they are transferred to wire cages in the same room. These cages are two feet square, and give practically no opportunity for exercise. We believe our experience abundantly establishes the fact that good growth can be obtained in severely restricted quarters.

In our earlier experiments, creek sand was used as a litter in both brooders and cages, but later this was replaced by pine shavings. The brooders and cages were cleaned once a week and supplied with new litter.

Rations.—The exact compostition of the rations and some additional description will be given in the appendix. Suffice it for the present to say that in some cases "synthetic" rations were used entirely, while in others some natural foodstuffs were incorporated in the synthetic rations. The synthetic rations were composed of relatively pure constituents, as protein, carbohydrates, fat, and mineral mixtures. Vitamin B was supplied in the form of dried yeast or a commercial concentrate.

There is always a question as to the freedom of nutrients from undesirable contamination, so we are including a brief description of the more questionable products, also of the salt mixtures.

Casein:—A commercial product obtained from Lister Bros., New York City. In previous feeding trials with rats, this product was found to be practically free from both vitamin A and vitamin B. In some cases the casein was used as purchased, and in others which will be desig-

nated, it was used only after it had been extracted continuously for one week with dilute acetic acid by the well known procedure of McCollum.

Corn Starch:—Buffalo Brand, purchased on the local market.

Butter Fat:—Fresh butter was obtained from the University Dairy, melted and filtered.

Cod Liver Oil:—Squibb preparation.

Dried Yeast:—Obtained from the Harris Laboratories, Tuckahoe, New York.

Vitamin B Concentrate:—Obtained from the Harris Laboratories, in both powder and tablet form. The tablets commonly used contained 50 mgm. of the vitamin concentrate and 50 mg. of starch.

Salt Mixture:—Mixtures of Chemically Pure salts in the following ratio:

SALT MIXTURE No. 14

Ca lactate	48.90%
K_2HPO_4, $3H_2O$	29.30%
NaCl	12.80%
Na citrate, $2Na_3C_6H_5O_7$, $11H_2O$	3.23%
Fe citrate, $Fe_2(C_6H_5O_7)_2$, $6H_2O$	2.44%
M_4SO_2, $7H_2O$	3.32%
NaI	0.11%

SALT MIXTURE No. 14A

Our number for Osborne and Mendel's* mixture.

SALT MIXTURE No. 14B

Osborne and Mendel's mixture	99.50%
Sodium Silicate	0.50%

Salt mixture No. 14 was used in all rations not designated otherwise.

The rations were made up at weekly intervals in order to prevent a decrease in the vitamin potency of the cod liver oil. The chicks were supplied at all times with fresh water from the University well, and a frequent supply of grit was furnished as crushed limestone and oyster shells.

The various lots of chicks are designated by the number of the ration which they received. For example, the lot receiving ration 167 is designated as Lot 167.

Weights.—The chicks were weighed when transferred to the brooder, and at weekly intervals thereafter. Our normal growth curve is taken from data published by Buckner, Wilkins, and Kastle.[12] They have compiled two sets of weights, one of chicks grown in confinement (as were ours), and one of chicks running at large with a hen. The chicks

*J. Biol. Chem. XXXIV, 309, 1918

in confinement grew more slowly than the others, and we have used them as our "normal", as we are not certain that the maximum rate of growth can be attained under such conditions.

Photographs.—For the purpose of comparing the various lots, and the individual chicks at different stages of growth, representative specimens from each group were usually photographed at the age of 40 days and again at the end of 101 days. Other photographs were taken at such times as it was believed they would be helpful in interpreting data.

ARRANGEMENT OF DATA

For the purpose of discussion, we have grouped the various lots of chicks into three groups. The first group is composed of those lots receiving the synthetic rations, the second of those receiving some natural food stuffs incorporated in the synthetic ration, and the third of those receiving various fractions of those natural food stuffs incorporated in the synthetic ration.

SYNTHETIC RATIONS

Our basal synthetic ration was composed of casein, salt mixture, cod liver oil, Crisco, starch, cellulose, and yeast, but during the course of the investigation, a number of changes were made in the proportions of its constituents. In order to compare agar with cellulose as a source of roughage, the cellulose was replaced by agar in the rations which were fed to three lots of chicks. To determine the adequacy of casein as a source of protein for growing chicks, a portion of the casein in one ration was replaced by an equal amount of meat protein.

On July 11, 1924, three lots of seven chicks each were placed on "synthetic" rations. Two lots received identical rations, containing cellulose as a source of roughage. Their treatment differed in that one was exposed to direct sunlight for two hours daily on all clear days except Sundays. The purpose of this variation was to determine the adequacy of the fat-soluble vitamins in the rations. The third lot was placed on a similar ration in which agar was the source of roughage.

In less than two weeks some of the chicks developed "leg weakness." Plimmer and Rosedale[13] have previously shown that the chick has a very high requirement for vitamin B, and that a deficiency of this factor produces leg weakness. The symptoms in a few of our cases indicated polyneuritis, but inasmuch as the same ration had been previously used with no indication of neuritic symptoms, we were slow to believe this difficulty was due to a deficiency of vitamin B. The symptoms were so suggestive, however, that we tried feeding additional yeast. Two chicks on the basal ration began in the second week to show symptoms of leg weakness, and on the thirteenth day they were badly affected. A small quantity of dried yeast (not weighed) was made into a thin paste and

each affected chick was given 1 cc. of the yeast emulsion daily for three successive days. Both had then recovered.

As soon as it was reasonably certain that the additional yeast had improved the condition of these chicks, all rations in use at this time were changed (July 26) by increasing the yeast from 6 to 8 per cent, and decreasing the carbohydrates correspondingly.

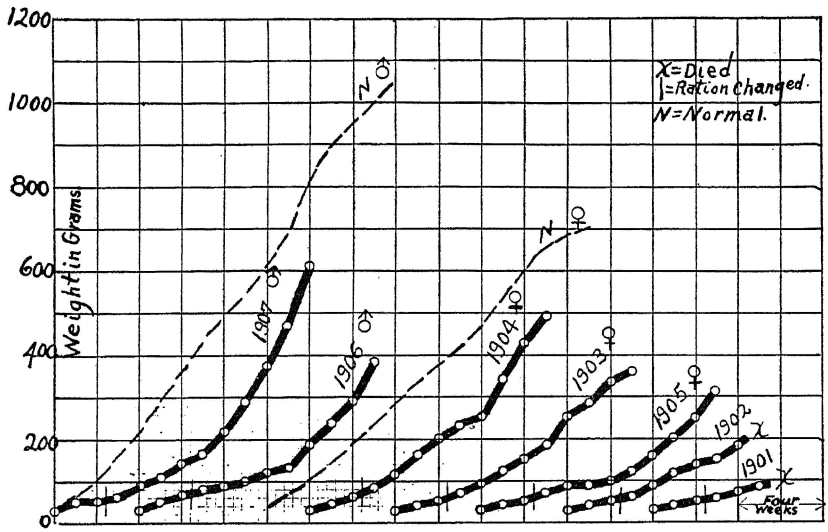

Fig. 1.—This group received ration 167 for the first seventeen days, but was then changed to Ration 241. Ration 167 was composed of casein 20, starch 52, yeast 6, Crisco 10, cod liver oil 5, salts 4, and cellulose 3. Ration 241 was similar in composition, but contained 8 per cent of yeast and 50 per cent starch. The form of the curve does not indicate that increasing the yeast had any effect on the rate of growth.

One chick from the lot described in Fig. 1, died on Aug. 18 without showing special symptoms, but when found dead it was outstretched in a manner suggesting polyneuritis. A second specimen died Sept. 6. It had been very thrifty previously, and on autopsy it was found to have a perforated crop, and sand had escaped into the abdominal cavity.

As shown in Fig. 2, there was no value in exposing chicks receiving this ration to direct sunlight. The history of this group was very similar to that of the lot receiving the basal ration alone, and three of the group ultimately died.

The third lot which was started at this time received the agar ration. The results obtained from this lot were very unsatisfactory, six of the birds having died, apparently from leg weakness, before the tenth week. On Sept. 20, the seventh chick became very weak. Since the symptoms seemed to suggest a deficiency of vitamin B, it was given an additional

quantity of yeast. There was a definite response to this addition, as may be observed in Fig. 3.

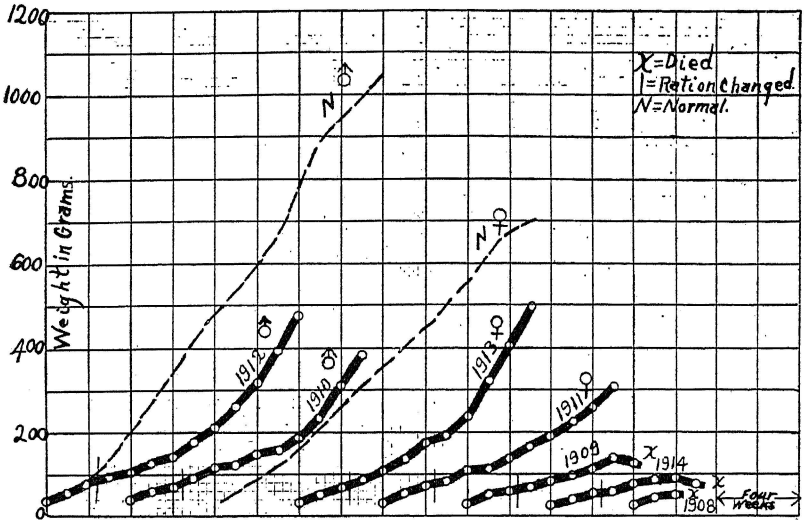

Fig. 2.—This group received the same ration as the one indicated in Fig. 1. When the weather permitted however the chicks were exposed to direct sunlight for two hours daily. The results indicate that the well-being of these chicks was not be improved by sunshine.

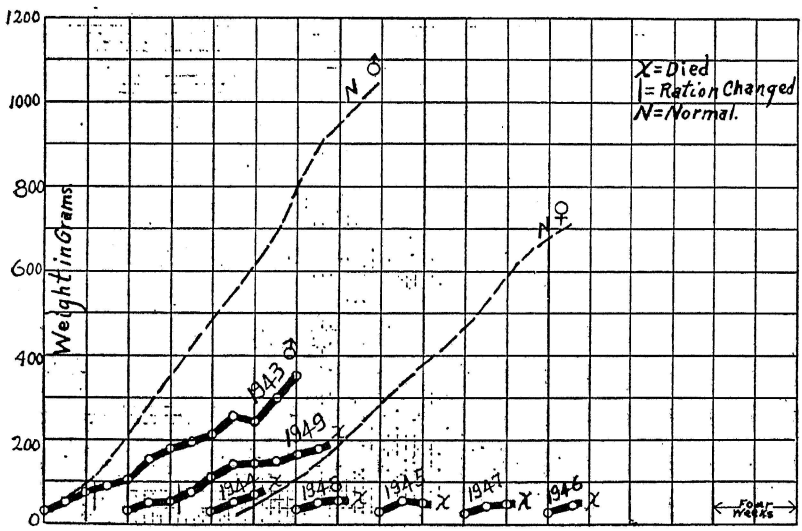

Fig. 3.—This group received the same ration as the one indicated in Fig. 1, except the cellulose was replaced by agar. Growth was very unsatisfactory and only one chick was alive when the feeding trial terminated. Chick 1943 appeared very nervous and unable to stand on Sept. 20. The yeast in the ration was increased to 10 per cent and there appeared to be a definite response to this addition as the chick regained its activity, and made marked gains in weight during the two weeks that followed.

Several cases of leg weakness had developed, due to a lack of vitamin B, so it seemed possible that this vitamin deficiency may have been the cause of the poor growth rate observed. In order to test that point another lot of 10 chicks, Sept. 15, 1924, was started on basal ration No. 241. Five chicks of this lot received however additional vitamin B in the form of the commercial concentrate. It will be recalled that Ration 241 contained 8 per cent of dried yeast, but other chicks under observation at this time, also receiving rations containing 8 per cent yeast, developed leg weakness. For that reason the ration for all ten of the chicks was changed by increasing the yeast to 10 per cent. The five mentioned however received additional vitamin B concentrate, but inspection of Fig. 4 indicates that the addition did not improve the ration in any way. The rate of growth was subnormal in all cases, and the chicks were very similar in appearance to those previously receiving this ration. Chick 3769 died on Dec. 5, and post mortem examination revealed the liver to be greatly enlarged and covered with numerous white spots.

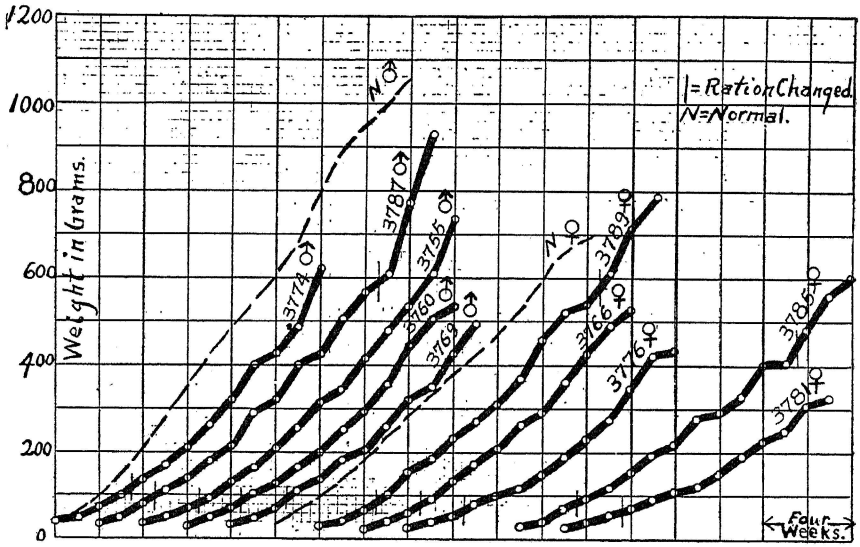

Fig. 4.—All chicks were started on the basal diet, No. 241, but Nos. 3787, 3785, 3789, 3776, and 3781, were each supplied in addition 40 mgm. of the Harris-Vitamin powder daily, in the form of the tablet preparation. This addition did not however have any perceptible effect. The yeast component was increased on the sixteenth day from 8 to 10 per cent of the ration, but we do not believe the change affected the rate of growth. At the termination of the feeding trial proper three of the chicks, Nos. 3787, 3789, and 3785 were changed to Ration 259, in which polished rice was substituted for the corn starch of the basal diet. It is evident that the retarded growth earlier in life was due to a defective diet.

In order to determine the permanency of the retarded growth among this lot, three chicks, 3785, 3787, and 3789 were placed on Ration 259

on Dec. 10. This ration contained polished rice, and was proving very satisfactory in another trial under way at this time. This change was followed by a marked increase in the rate of growth of these chicks during the few weeks in which the feeding trial was continued.

The feeding trial we have just described was initiated in order to determine whether or not our basal ration was deficient in vitamin B. At the same time another possibility was suggested, the amino acid content of the casein may have been partially inadequate. For that reason another group was placed on a ration very similar to the basal diet, except 5 per cent of the casein had been replaced by meat protein.

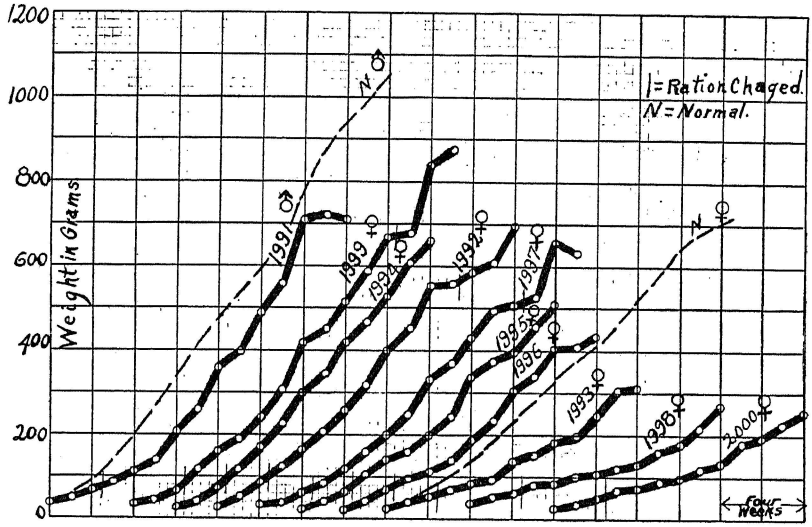

Fig. 5.—This group received Ration 247, which was composed of casein 15, meat protein 5, starch 50, cod liver oil 5, Crisco 10, yeast 8, salts 4, and cellulose 3. On the sixteenth day the yeast was increased to 10 per cent. In some cases the rate of growth seemed improved by substituting 5 per cent meat protein for an equal quantity of casein. We are not certain however that the difference is significant. Nos. 1998 and 2000 had definite attacks of leg weakness. Three chicks, Nos. 1999, 1992, and 1997 were kept under observation somewhat longer than the others, and the ration changed by substituting meat protein for all of the casein. Apparently the amino acid mixture of the ration was not improved by the change.

The meat protein used in this investigation was obtained from pork muscle. The lean pork was ground in a meat chopper, and dried in a current of air. When dry it was thoroughly extracted with each of the following solvents, ether, 95 per cent alcohol, 70 per cent alcohol, and hot water. It was then dried, reground and incorporated in the ration as desired.

A few cases of leg weakness appeared among the lot receiving this ration, and the yeast was increased from 8 to 10 per cent. This increase was not sufficient however to entirely relieve the symptoms of leg weak-

ness, and growth was considerably below normal. We are convinced that the unsatisfactory growth obtained with our basal ration was not due to inadequacy of the protein.

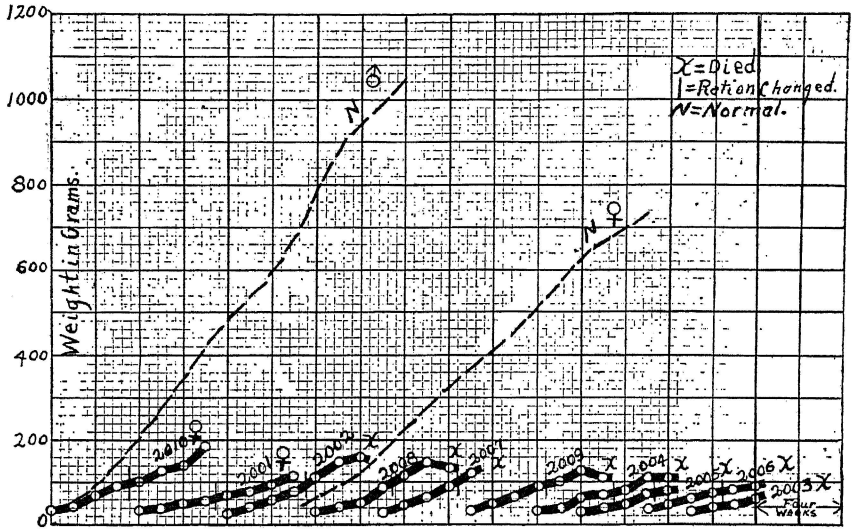

Fig. 6.—This group received ration 226, which was composed of washed casein 20, yeast 10, Crisco 10, starch 53, cellulose 3, and the unsaponifiable residue from 5 parts of cod liver oil. The yeast was increased to 15 per cent on the twenty-fourth day due to the appearance of leg weakness among the lot. The change appeared to have no beneficial effect, as nine of the chicks had died before the end of the seventh week.

On Nov. 10 a lot of ten chicks was placed upon a modified form of our synthetic ration, because of the success of Hart and collaborators with one of similar composition. In this diet the fat-soluble vitamins were supplied as the unsaponifiable residue of cod liver oil, equivalent to 5 per cent of the original product. Due to the appearance of leg weakness, the amount of yeast was increased to 15 per cent on Dec. 4, but despite the change, growth proved very unsatisfactory. In fact only one chick was alive at the end of the seventh week, when it weighed 115 grams. Post mortem examination of eight of the nine chicks which died, revealed the following: Crop filled with cellulose, heart covered with a whitish film, kidneys with spots on the surface, urethas distended, and the intestines blood-shot.

A second lot of ten chicks (Fig. 7) was started Nov. 10, on a ration similar to the one just described, but with agar substituted for cellulose as a source of roughage. The fat-soluble vitamins were in this case also, supplied as the unsaponifiable residue of cod liver oil, and the yeast was increased to 15 per cent on Dec. 4. The history of the two lots

receiving the unsaponifiable residue was practically identical, and in each case there was only one chick alive at the end of the seventh week. The post mortem findings were the same in both lots, and as yet we have no adequate explanation for the results noted.

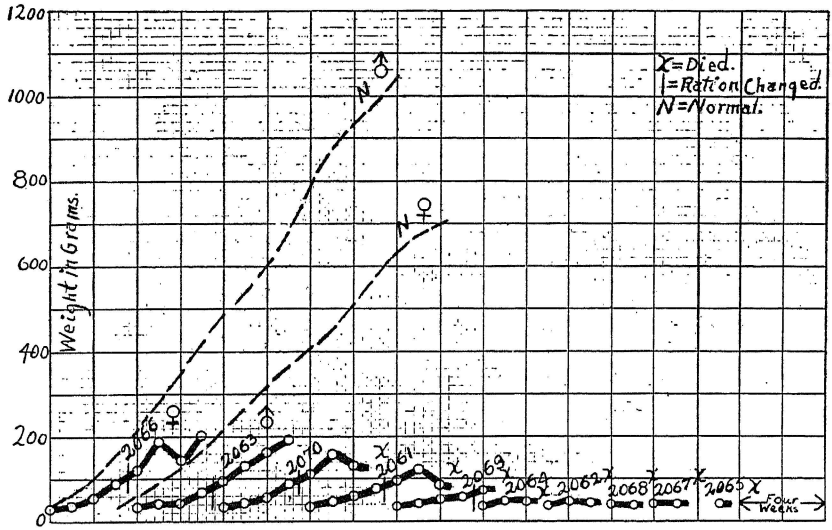

Fig. 7.—This group received the same rations as the group in Fig. 6, except the cellulose had been replaced by agar. The substitution apparently had no beneficial effect as only one chick was alive at the end of the seventh week.

Since our second group of chicks, receiving roughage in the form of agar, had met with complete failure, we decided to repeat the feeding trial with a slightly different ration. A lot of ten chicks (Fig. 8) was placed on such a ration (292), on Feb. 24, 1925. The vitamin B was supplied daily in the form of Harris tablets, and each chick received one daily until the eighteenth day when the number was increased to three, due to the appearance of leg weakness among the lot. On March 16, the supply of tablets was temporarily exhausted, and we incorporated 15 per cent of dried yeast in the diet as the source of vitamin B. The chicks continued on this diet until March 24, when they were transferred to ration 309. This was similar to ration 292, except 6 per cent of starch had been replaced by an equal amount of yeast, and salt mixture No. 14 B had been substituted for No. 14 A. In addition, each chick received two vitamin tablets daily until the feeding trial terminated. Though growth among this lot was unsatisfactory, it was our most successful feeding trial with an agar ration. Seven chicks were alive at the end of the eighth week

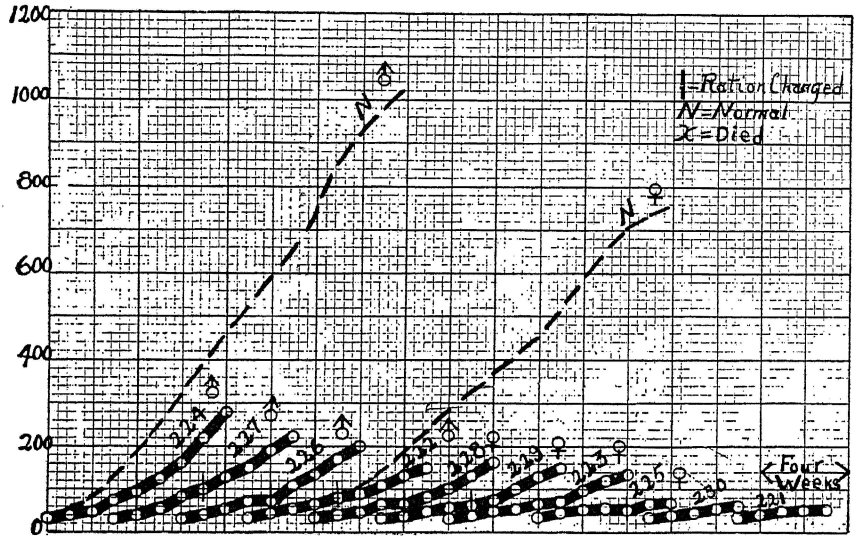

Fig. 8.—This group received Ration 292, which was composed of washed casein 20, Crisco 10, cod liver oil 5, salts 4, agar 3, starch 58, and vitamin tablets. Due to the appearance of leg weakness among the lot, 6 per cent of the starch was replaced by yeast on the twenty-fourth day. Though growth was subnormal and feathering was poor, seven of the chicks were alive at the end of the eighth week. The results indicate that agar may be used as a source of roughage when the ration contains an adequate supply of vitamin B.

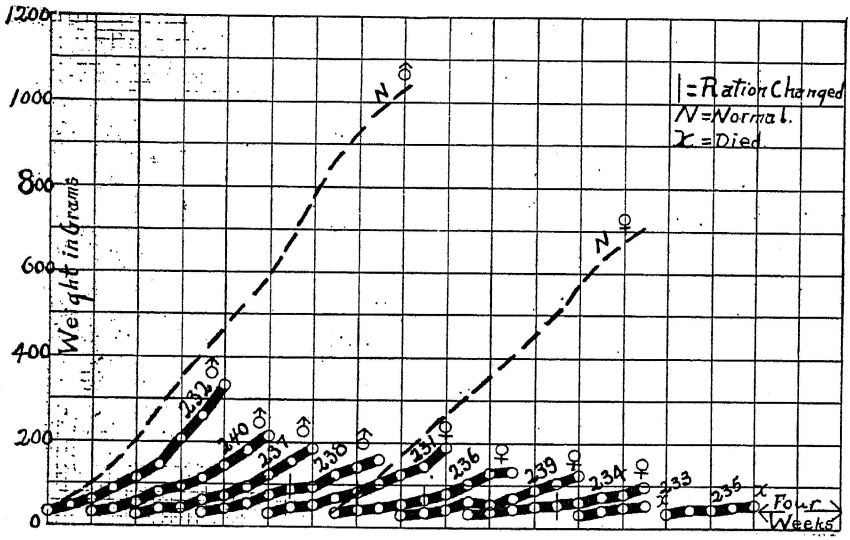

Fig. 9—This group received Ration 293, which was similar to Ration 292, except the agar was replaced by cellulose. Six per cent of the starch was replaced by dried yeast on the twenty-fourth day. Though growth was subnormal and symptoms of leg weakness were frequently observed, eight chicks were alive at the end of the eighth week.

when the feeding trial terminated, three having died from leg weakness. The history of this lot was almost identical with that of a lot receiving the basal ration of this series (Fig. 9).

ADDITION OF NATURAL FOOD STUFFS

At the time we were conducting feeding experiments with synthetic rations, we also turned our attention to the effect of incorporating certain natural food stuffs in our synthetic diets. The natural food stuffs used for this purpose were egg yolk, polished rice, and ox liver.

The egg yolk was prepared by coagulating the yolks with alcohol, drying over a hot air bath, and grinding in a meat chopper.

The polished rice was obtained on the local market, and ground to a powder before incorporating it into the ration.

The ox liver was purchased on the local market, ground in a meat chopper, dried in a current of warm air, and reground before being added to the ration.

On July 12, 1924, two lots of seven chicks each were placed on a ration containing the natural food stuffs. One lot received a ration similar to our basal synthetic ration in use at this time, but differing only in that part of the casein and Crisco had been replaced by 10 per cent of dried egg yolk. The other lot received a ration in which the starch and part of the casein of our synthetic ration had been replaced by polished rice. The composition of the rations was practically the same, as judged by the routine analysis.

On July 28, after leg weakness had appeared in some of the other lots, the yeast was increased from 6 to 8 per cent in these two rations. From the standpoint of health and vigor, the lot receiving the egg yolk (Fig. 10) was entirely satisfactory. The rate of growth was approximately normal, and no chicks howed signs of leg weakness during the eleven weeks they were under observation. All, however, exhibited the peculiar ruffled condition of the feathers that seems so characteristic of chicks reared on synthetic diets.

The history of the lot on the rice ration (Fig. 11) was fairly satisfactory but not completely so. On July 31, one individual developed leg weakness though it was then receiving a ration containing 8 per cent of dried yeast. This chick was not a White Leghorn, but one of a dark

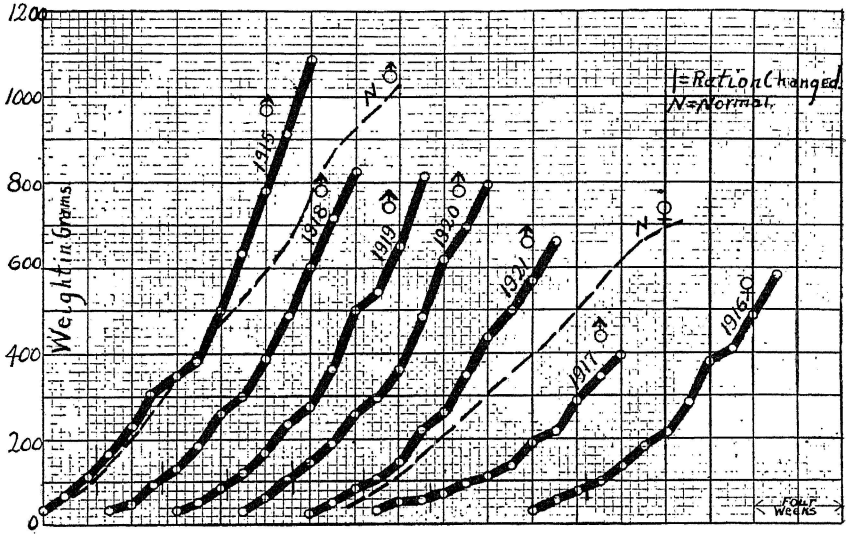

Fig. 10.—This group received Ration 195, which was composed of casein 16.8, starch 52, Crisco 3.2, yeast 6, cellulose 3, salts 4, cod liver oil 5, and dried egg yolk 10. The egg yolk was dried and incorporated in the ration, and the substitution resulted in a marked acceleration of the rate of growth. Fig. 1 should be consulted for a comparison. The ration was changed to No. 244 at the point indicated, in order to increase the supply of yeast.

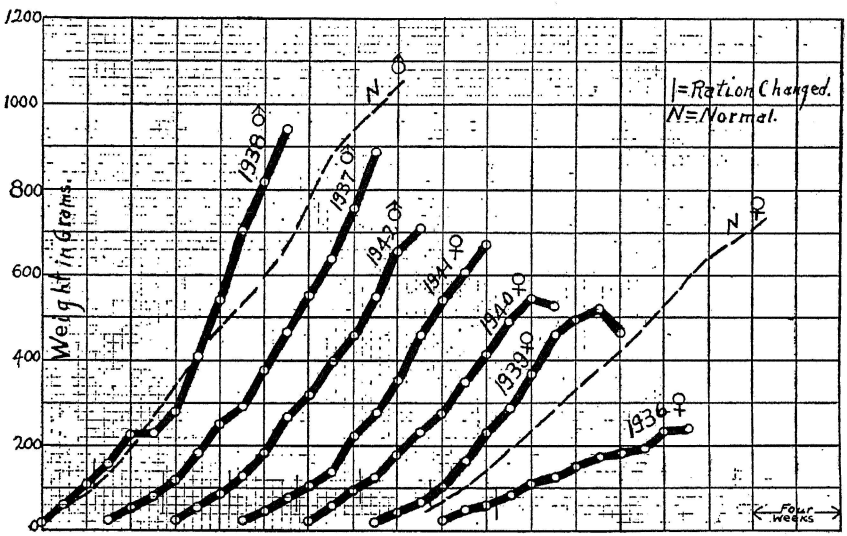

Fig. 11.—This group received Ration 237, which was composed of casein 16, polished rice 56, Crisco 10, yeast 6, cod liver oil 5, salts 4, and cellulose 3. This illustrates the favorable effect on growth of substituting polished rice for corn starch. These chicks were also feathered normally. Chick 1936 developed "leg weakness" on the twentieth day, but survived until the feeding trial closed. The increase of yeast on the seventeenth day did not seem to affect the rate of growth. Chicks Nos. 1939 and 1940 developed roup about two weeks before the end of the feeding trial. The yeast component was increased to 8 per cent at the point indicated.

breed which had been hatched in the same incubator, due to a mistake in the selection of the eggs. The ration was not changed and the bird did not entirely recover, though it survived the eleventh week, when the feeding period ended. Two pullets developed roup, and were treated with antiseptics in an effort to effect recovery. The treatment was ineffective, but the birds lived until Sept. 27, when the feeding trial terminated. With one exception, the rate of growth was normal, and in all cases the feathers were in good condition. The pigmentation of the skin was light, undoubtedly due as Palmer and Kempster[14] have shown to the lack of pigment in the diet.

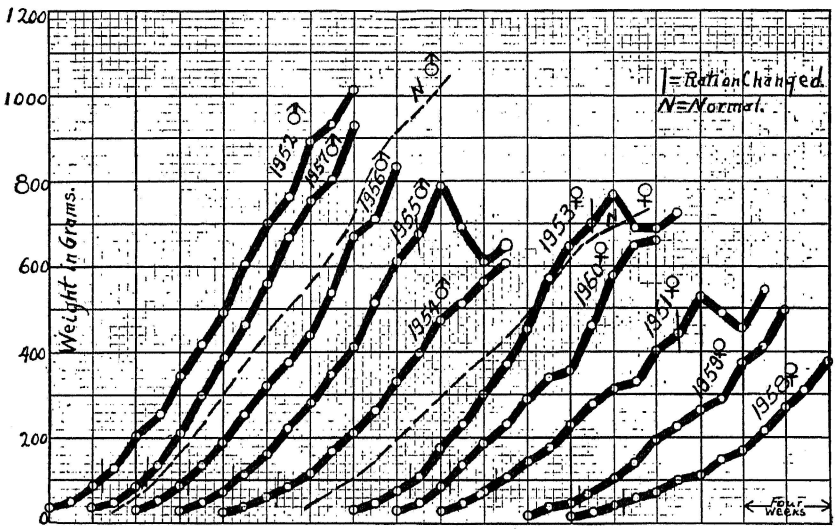

Fig. 12.—This group received Ration 244, which was composed of casein 16.8, corn starch 50 Crisco 3.2, cod liver oil 5, dried yeast 8, cellulose 3, salts 4, and dried egg yolk 10. On the sixteenth day the yeast was increased to 10 per cent. This chart again illustrates the generally favorable effect of incorporating dried egg yolk in the ration. The egg yolk itself undoubtedly increased the supply of vitamin B but one chick, No. 1959, had a persistent case of leg weakness. The symptoms were first noted on Nov. 4 at the age of 50 days, and one feeding of 40 mgm. of the Vitamin-Harris concentrate, effected a temporary recovery. Eight days later the chick had a relapse, and was fed yeast emulsion continuously from Nov. 13 to Dec. 8. During this time the chick was alert and seemingly in good health, except for the deformity of the legs.

On Sept. 15, another lot of ten chicks was placed on a ration containing egg yolk. This ration was identical with that used in the preceding series just described, but the diet was changed on Oct. 1, due to the appearance of leg weakness in some of the other lots. This change

consisted in increasing the yeast from 8 to 10 per cent, and decreasing the starch a corresponding amount. This group grew at about the same rate as the previous lot, and there were no mortalities. A case of leg weakness developed on Nov. 4, but a single feeding of additional vitamin concentrate was sufficient to effect a temporary recovery and no more was given at that time. On Nov. 21, the chick was again in bad condition, and the feeding of yeast emulsion was begun. General recovery was immediate, and this specimen gained rather rapidly in weight, but never regained control of its legs.

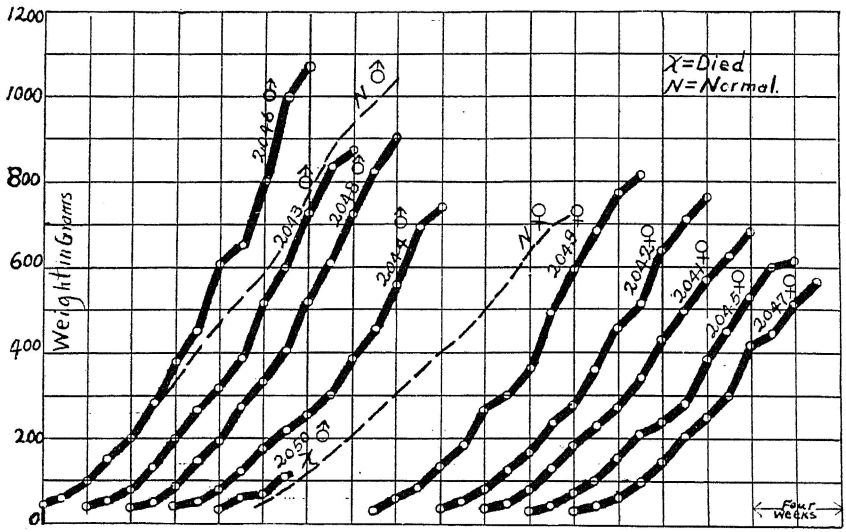

Fig. 13.—This group received Ration 230, which was composed of dried liver 15, meat protein 6, yeast 10, salts 4, cellulose 3, cod liver oil 5, Crisco 10, and starch 47. This proved to be one of our most successful diets. Growth and feathering were normal. Chick 2050 died on Dec. 5 from an unknown cause.

On Nov. 10 a lot of ten chicks was placed on a ration (Fig. 13) containing dried liver. It is to be noted that this ration contained 15 per cent of dried ox liver and 6 per cent of meat protein as a source of protein. This proved to be one of our most successful rations. Growth was excellent, and the feathering was normal. One chick, No. 2050, died on Dec. 5. It had shown no previous symptoms of being sick, and post mortem examination revealed no visible cause of death. Chick No. 2047 was killed on Jan. 30 for examination, but the remainder of the lot thrived until the feeding trial was terminated on Feb. 2.

ADDITION OF THE FRACTIONS OF NATURAL FOODSTUFFS

Since the addition of natural foodstuffs to our synthetic rations had proven so beneficial, we became interested in fractionating these natural foodstuffs and incorporating the various fractions in our rations. It seemed evident that our basal diet was deficient in some substance carried by the natural foodstuffs, and so we hoped to separate the active portion, and thus make possible its identification. The natural foodstuffs fractionated were polished rice, dried egg yolk, and dried liver. The separation was accomplished by extracting the dry material with various solvents.

The polished rice was first ground very finely, and then extracted for a period of one week with dilute acetic acid. The total extract was evaporated to a small volume on a water bath, and then to dryness in a desiccator. The residue thus obtained constituted less than one per cent of the original material. The two fractions were then preserved until incorporated in the ration.

Both the dried egg yolk and dried liver were consecutively extracted with ether, 95 per cent alcohol, 70 per cent alcohol, and hot water, as long as a trace of material was being removed by the solvent. The water extracts were discarded. The ether extract was reduced to a small

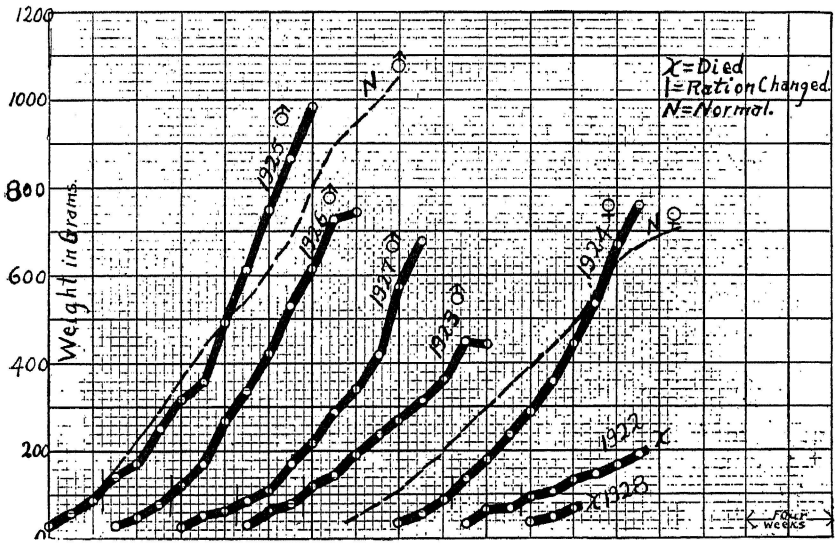

Fig. 14.—This group received Ration 196, which was composed of casein 16.8, starch 52, Crisco 10, yeast 6, salts 4, cod liver oil 5, cellulose 3, and yolk protein 3.2. On the fourteenth day the yeast was increased to 8 per cent, after leg weakness had appeared among the lot. Only five of the chicks were alive at the end of the eleventh week, and the rate of growth was uneven though considerably better than shown by those receiving the basal ration (Fig. 1). The yeast component was increased at the point indicated.

volume and preserved. The two alcohol extracts were combined and reduced to a small volume. Of the eight fractions thus obtained, two from polished rice, two from egg yolk (the alcohol extracts were not used) and three from dried liver, were incorporated in our synthetic rations, at the same time making necessary adjustments to keep the protein and fat of the diets constant.

Our first feeding trial with a ration of this type was begun on July 12, 1924, when a group of seven chicks was placed on a synthetic ration (Fig. 14) in which part of the casein was replaced by yolk protein. The amount of yolk protein was equivalent to that obtained from 10 per cent by weight of dried egg yolk. The yeast was increased from 6 to 8 per cent on July 28, due to the appearance of leg weakness in some of the other lots. Chick 1928 died on July 28 from an unknown cause. Chick 1922 showed first symptoms of leg weakness on Aug. 6. A severe case of leg weakness followed and continued until the chick's death on Sept. 11. The five remaining birds showed no signs of leg weakness during the eleven weeks they were on experiment, but growth was unsatisfactory in some cases and the chicks developed the typical ruffled feathers.

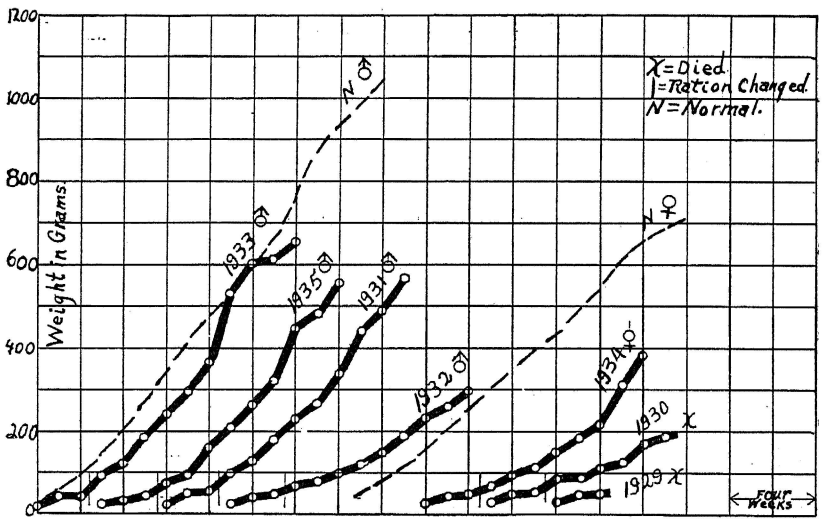

Fig. 15.—This group received Ration 197, composed of casein 20, starch 52, Crisco 3.2, yeast 6, salts 4, cellulose 3, cod liver oil 5, and yolk lipins 6.8. Leg weakness appeared among the lot on the fourteenth day and the amount of yeast was increased from 6 to 8 per cent. Chick 1929 died on July 25, and Chick 1930 died on Sept 7, both deaths being due to leg weakness. Growth was subnormal and the feathering poor. The addition of yolk lipins did not seem to improve the diet.

On the same day a similar lot (Fig. 15) was started on a ration containing the yolk lipins obtained from 10 per cent by weight of dried egg yolk. For the reasons already given the yeast was increased from 6 to

8 per cent on July 28, but Chick 1919 had died of leg weakness on July 25. On the same day Chick 1930 showed symptoms of the disease, and was given 1 cc. of yeast emulsion daily for three consecutive days. At the end of this time it appeared very much relieved. The symptoms reappeared on Aug. 5, and an advanced case of leg weakness soon developed. We were unable to afford any relief and the chick died on Sept. 7. The remaining chicks of this lot showed no symptoms of leg weakness during the period of the feeding trial, but the rate of growth was very unsatisfactory.

It will be recalled that when the egg yolk was incorporated in the ration, approximately normal growth resulted. When an equivalent amount of the yolk lipins was substituted for Crisco, however, the ration was not improved in the slightest degree. The substitution of yolk protein for casein gave markedly better results than the basal diet, but growth was still unsatisfactory. No explanation suggested itself, so the trials were repeated but the yolk protein was probably subjected to a more thorough extraction in the second trial. We believe that this more complete extraction explains the fact that in subsequent trials, the yolk protein alone, as well as the lipins alone, failed, when incorporated in the ration, to improve it.

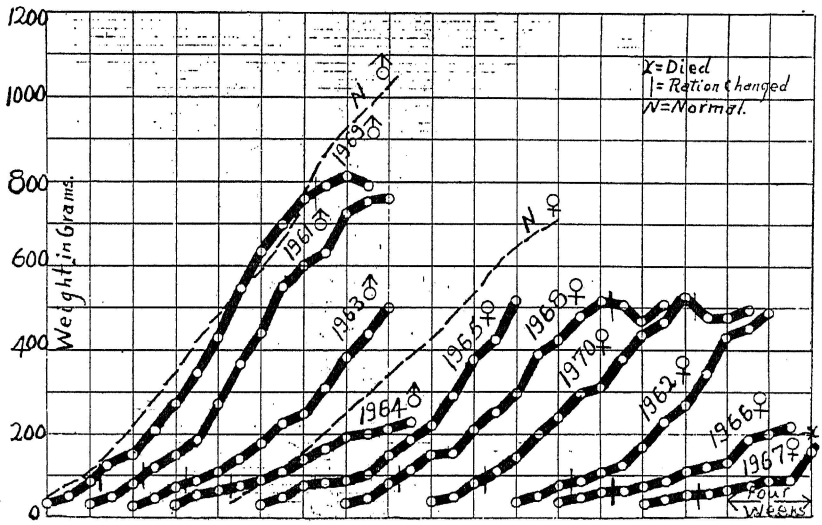

Fig. 16.—The ration, No. 245, fed this group is very similar to that described in the legend to Fig. 14. Yolk protein, 3.2 per cent, was substituted in the basal diet for an equal quantity of casein. Some cases of leg weakness developed and the yeast was increased to 10 per cent on the fifteenth day. Chick 1957 died of leg weakness on Nov. 8. Chick 1966 developed a severe case of the disease on Nov. 13, and died Dec. 8. Comparison with Fig. 14 shows that growth was much less satisfactory than in the earlier trial.

As mentioned in the text, the ration of three of the birds was changed near the end of the trial, by substituting liver lipins for part of the Crisco. The substitution did not improve the ration.

Two groups of ten chicks each were used in the second trial, begun Sept. 15. One group (Fig. 16) received the yolk protein, the other (Fig. 17) the lipins, incorporated in the ration. These rations contained 8 per cent of dried yeast as the source of vitamin B, but on Oct. 1, after a number of cases of leg weakness had appeared among the lots, the yeast was increased to 10 per cent.

Three chicks, Nos. 1964, 1966, 1967 from the group receiving the yolk protein developed leg weakness and were given 40 mgm. vitamin B concentrate daily for more than 30 days. At the end of this time No. 1964 was apparently well, but Nos. 1966 and 1967 seemed but slightly improved. Neither of the two recovered from the disease. No. 1967 died on Nov. 8, and 1966 died one month later. As a whole, growth of this lot was unsatisfactory, much more so than in the previous trial.

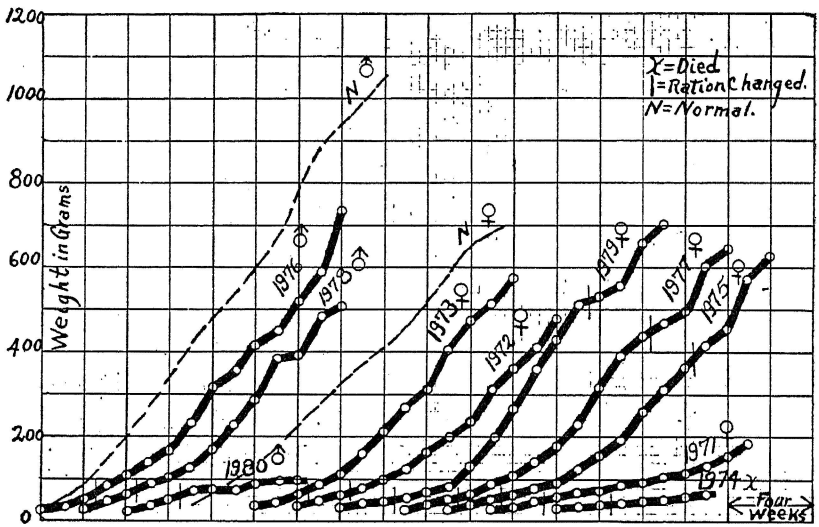

Fig. 17.—This group received Ration 246, which was composed of casein 20, starch 50, Crisco 3.2, yeast 8, salts 4, cod liver oil 5, cellulose 3, and yolk lipins 6.8. On the sixteenth day the yeast was increased to 8 per cent, after leg weakness had appeared in some of the lots. Chick No. 1974 died on Nov. 9, and Chick 1980 died on Nov. 14, both deaths being due to this disease. Growth was subnormal and the feathers were in poor condition. Chick No. 1978 developed a case of leg weakness on Nov. 10 and was given yeast emulsion daily until Dec. 8 when the feeding trial terminated. It never regained use of its legs, but it continued to grow until the last. Toward the end of the period, three of the chicks were transferred to Ration 256. This was very similar to Ration 246 but 3.2 per cent of liver protein had been substituted for an equal quantity of casein. We were not certain that this change improved the ration.

In order to study the nutritional properties of the liver lipins, three chicks of the above group, Nos. 1968, 1969, and 1970 were separated from the rest of the group, and placed on a ration similar in composition but with 6.8 per cent of Crisco replaced by liver lipins. As far as growth

was concerned the change was not beneficial, and we concluded that for our purpose liver lipins are inferior to egg yolk lipins.

Leg weakness first appeared among the lot receiving yolk lipins, on Oct. 2. The affected chicks were treated with vitamin concentrate, yet there remained symptoms of the disease among the group throughout the feeding trial. Chick 1974 died Nov. 9 and Chick 1980 died Nov. 14. The growth of the remaining chicks was very slow and was similar to that of the previous lot receiving the yolk lipins.

In order to gain some hint as to the nutritional properties of liver protein, Nos. 1975, 1977, and 1979 were separated from the rest of the group on Dec. 10 and placed on a similar ration, No. 256, in which part of the casein had been replaced by 3.2 per cent liver protein. During the three weeks which followed, the rate of growth of these three chicks appeared to have been slightly accelerated by the change.

For the purpose of studying the effect of our process of extraction upon the nutritive properties of dried egg yolk, a third group of ten chicks was placed, Sept. 15, on a ration containing both fractions, but in the same ratio as received by the preceding lots. In order to insure a more liberal supply of vitamin B, the yeast was increased to 10 per cent

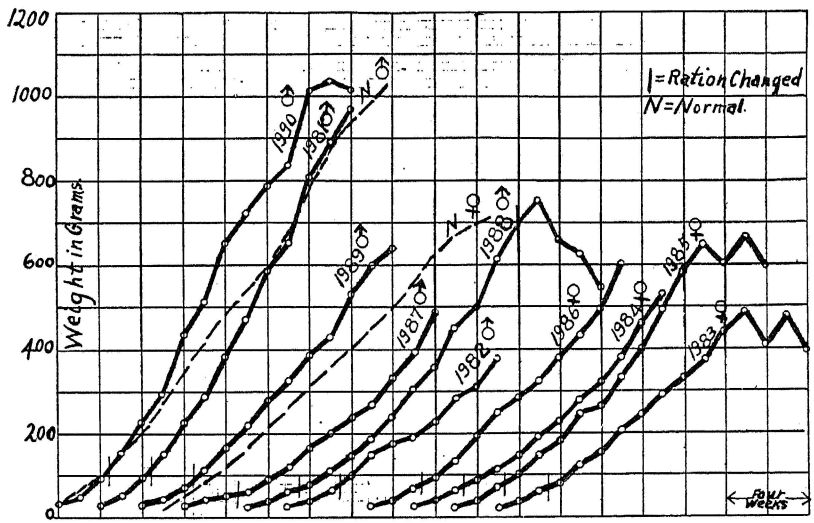

Fig. 18.—This group received Ration 243, Casein 16.8, yolk protein 3.2, starch 50, yeast 8, Crisco 3.2, cod liver oil 5, yolk lipins 6.8, cellulose 3, salt mixture 4. This diet contained both of the egg yolk fractions used. All of the chicks were alive at the end of the twelfth week, and the rate of growth was normal. The feathering was imperfect and the history of the group was very similar to the group receiving the whole egg yolk. It appears that the growth promoting factor was not affected materially by fractionating the egg yolk. At the point indicated Chicks 1983, 1985, and 1988 were transferred to the basal diet, No. 223. The result was a prompt loss of weight.

on Oct. 1, and there were no cases of leg weakness among this lot during the twelve weeks the chicks were under observation. The rate of growth was normal and the feathers were in fair condition. We concluded therefore that the unsatisfactory performance of the chicks receiving either of the single yolk fractions, was not due to destruction of any nutritional factor by the process of extraction. On December 1, chicks Nos. 1983, 1985, and 1988 were separated and placed on the basal ration then in use, No. 223. As far as the growth and well-being of the chicks were concerned, the outcome was very unsatisfactory. During the four weeks which followed all three birds lost weight and chick 1988 showed symptoms of leg weakness when the feeding trial was discontinued. The interpretation of this result must await further data.

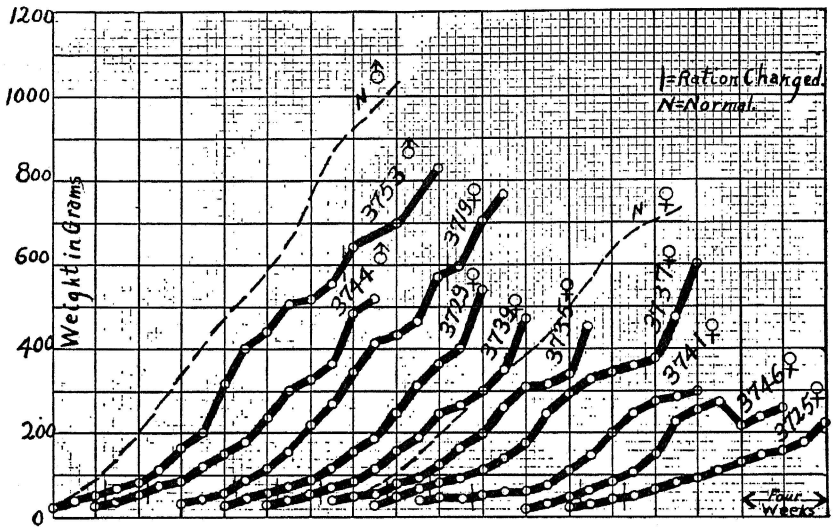

Fig. 19.—This group received Ration 248, which was identical with Ration 237 (Fig. 11) except the rice had been extracted for one week with dilute acetic acid. All of the chicks were alive at the end of the twelfth week when the feeding trial terminated. Although the appearance of the chicks was satisfactory, the rate of growth was subnormal. It appears that the active factor had been partially removed from the rice by the extraction. See Fig. 11 for a comparison with chicks receiving unextracted rice.

After our former ration containing polished rice had proven so satisfactory, we became interested in the nutritional properties of fractions we might obtain from it. The method of preparing these extracts is given on page 19. The residue of extracted rice was incorporated in Ration 248, and on Sept. 15 ten chicks (Fig. 19) were placed on this diet. Leg weakness appeared among them Oct. 1, making it necessary to increase the yeast from 8 to 10 per cent. On Oct. 7, all cases of leg weakness had recovered, and there were no recurrences during the twelve

weeks of the feeding trial. The rate of growth of this lot was below normal, and the general appearance of the chicks was unsatisfactory. On December 10, three chicks from this lot, Nos. 3719, 3737, and 3734 were separated and placed on another diet. This contained one per cent of the extracted material in addition to the rice residue, and during the three weeks following there was a marked acceleration in the rate of growth of these chicks. The rice extract undoubtedly contains a necessary nutrient, but we have no idea as to the nature of the active material.

It will be recalled that when either of the egg yolk fractions alone was substituted for similar constituents of the basal diet, the result was not satisfactory. When however the two fractions were combined in the same ration, normal growth resulted. No satisfactory explanation occurred to us, so the study was continued by combining the yolk fractions with other constituents. The combinations used were meat protein with yolk lipins, and yolk protein with milk fat. As a control, meat protein and milk lipins were also combined.

In accordance with the plan outlined therefore, a ration containing 3.2 per cent of meat protein and 6.8 per cent of yolk lipins (Fig. 20) was

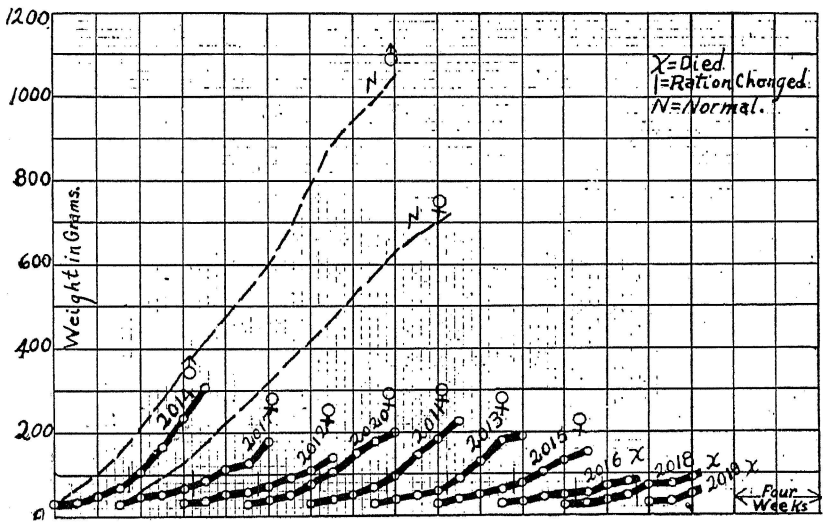

Fig. 20.—This group received Ration 227, which was composed of washed casein 16.8, meat protein 3.2, starch 48, Crisco 3.2, yolk lipins 6.8, yeast 10, salts 4, cellulose 3, and cod liver oil 5. On the twenty-fourth day the yeast was increased to 15 per cent, after a number of the chicks had shown symptoms of leg weakness. Chick 2015 appeared to be in a critical condition from leg weakness on Nov. 26. It was photographed, given a vitamin concentrate and photographed the following day, when it appeared vastly improved. The rate of growth of this group was subnormal and the general appearance of the chicks was poor. We interpret our observations as indicating that some factor other than protein inheres in the egg yolk residue. At any rate, muscle protein cannot be substituted successfully for yolk protein.

fed to ten chicks, beginning on Nov. 10. This may be regarded as a substitution of meat protein for yolk protein. Although this ration contained 10 per cent of dried yeast, leg weakness made its appearance on Nov. 26, and Chick 2019 died on Nov. 29 from this disease. Three other chicks in this lot were stricken with leg weakness but were cured by treatment with yeast emulsion. Photographs of Chick 2015, Figs. 35 and 36 will show a typical case before and after a cure. This feeding trial continued for seven weeks and growth was very unsatisfactory.

In order to estimate the nutritional value of milk fat, a ration containing 3.2 per cent yolk protein and 6.8 per cent milk fat was fed to ten chicks (Fig. 21) beginning Nov. 10. In this case we substituted the

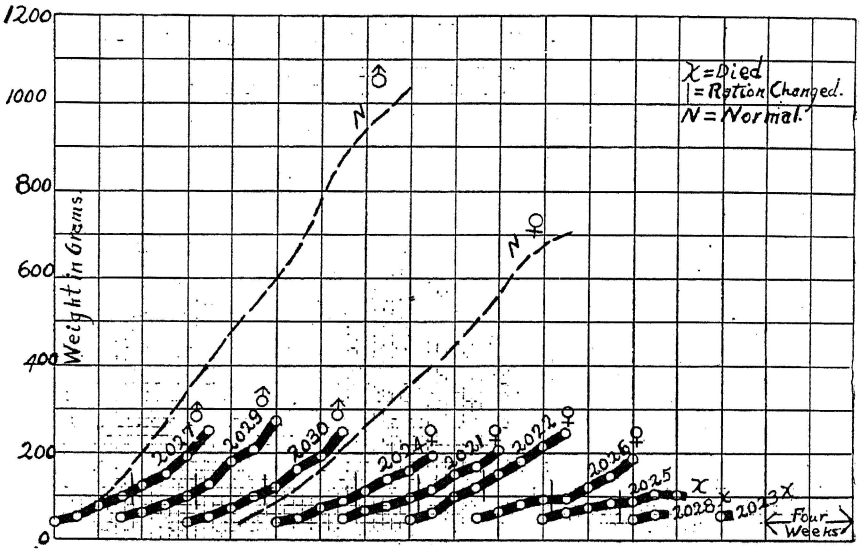

Fig. 21,—This group received Ration 228, which was composed of washed casein 16.8, yolk protein 3.2, starch 48, yeast 10, salts 4, butter fat 6.8, cod liver oil 5, and cellulose 3. Chick No. 2023 died on Nov. 15 and No. 2028 died on Nov. 17, both deaths resulting from an unknown cause. The yeast was increased to 15 per cent on the twenty-fourth day, but the growth and general appearance of the lot was very unsatisfactory. We were not successful in attempts to substitute milk fat for the yolk fats, and are at a loss to explain the nutritional value of the yolk lipins.

lipins of milk for the lipins of egg yolk. Chick No. 2023 died on Nov. 15 and Chick No. 2028 died on Nov. 17, neither showing any evident cause of death. However leg weakness appeared among the lot on Nov. 25 and it was necessary to increase the yeast to 15 per cent in order to insure an adequate supply of vitamin B. Chick 2025 never recovered from the attack of leg weakness, and died on Dec. 23. The remaining chicks continued to grow slowly until Dec. 29 when the feeding trial ended. The rate of growth was subnormal, and the chicks were poorly

feathered. The rate of growth and general appearance of this lot were less satisfactory than of the lot receiving the yolk lipins. This seems to indicate that the yolk lipins contain more of the growth promoting factor than the milk lipins.

Another group of this series was placed on a ration containing 3.2 per cent meat protein and 6.8 per cent butter fat. Leg weakness appeared among this lot on Nov. 25 and Chick 2040 died the same day. No. 2031 died on Nov. 28 and Nos. 2032 and 2034 died on Nov. 29, all dying apparently from leg weakness. As in other rations in use at this time the yeast was increased to 15 per cent on Dec. 4 in order to insure an adequate supply of vitamin B. This ration did not prove as satisfactory as

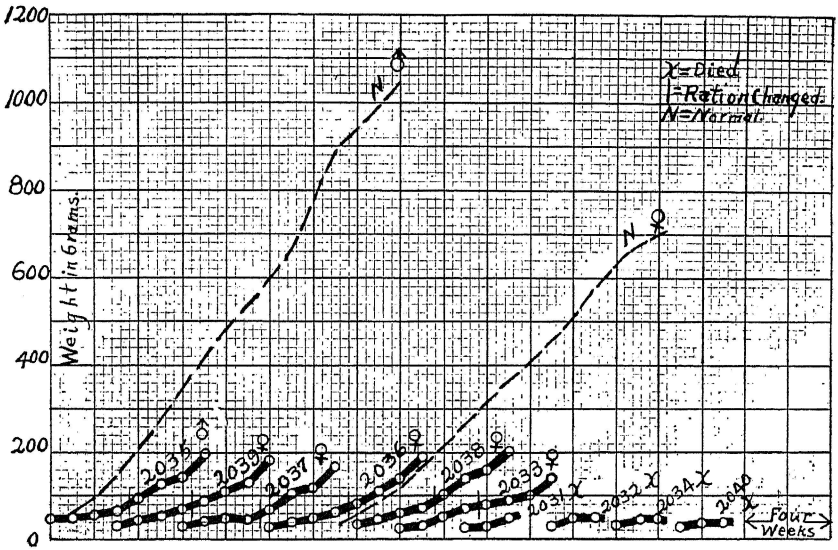

Fig. 22.—This group received Ration 229, which was similar to Ration 228 (Fig. 21), except meat protein was substituted for the yolk protein. Four of the chicks died before the end of the third week with leg weakness, consequently the yeast was increased to 15 per cent on the twenty-fourth day. The remainder of the chicks survived until the end of the feeding trial but the rate of growth was subnormal and the feathering poor. The substitution of muscle protein and milk fat for egg yolk protein and lipins resulted in nutritional failure.

any of those containing egg yolk fractions. Only six chicks were alive at the end of the seventh week. Growth was subnormal, and the feathering was unsatisfactory.

The explanation of the somewhat surprising results obtained with the egg yolk fractions must await further work. We have considered the possibility that the only factor concerned is palatability, but do not consider it probable. It does not seem reasonable that there

should be any marked difference between the palatability of egg yolk lipins and milk fat, or between the yolk protein and meat protein.

Somewhat later, on Nov. 10, our study of the rice fractions was continued by placing a group of ten chicks on a diet containing one per cent of rice extract. It is to be noted that this was similar to our basal ration, but one per cent of starch had been replaced by the rice extract. Chick 2059 developed leg weakness on Dec. 28, from which it never recovered, although it lived until the day before the feeding trial terminated and gained slightly in weight. Since the supply of vitamin B seemed insufficient, the yeast was increased to 15 per cent on Dec. 4.

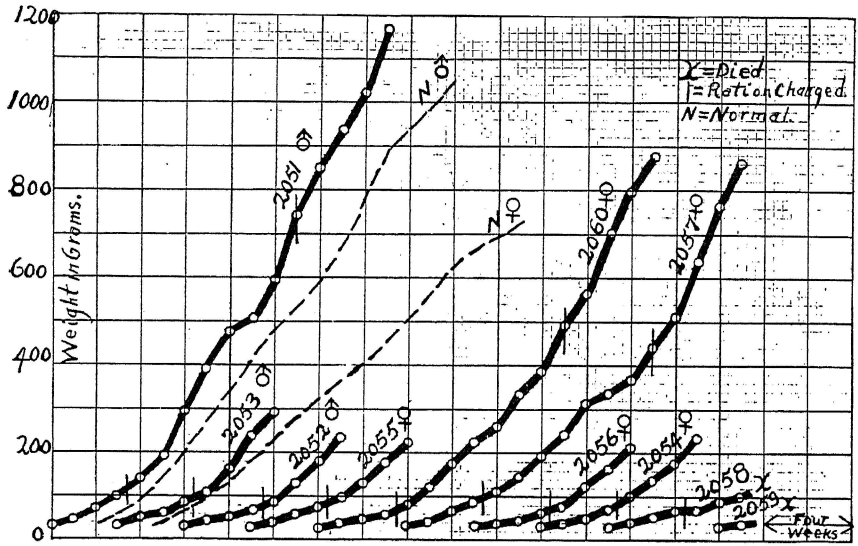

Fig. 23.—This group received Ration 231, which was similar to our basal ration, except one part of the starch had been replaced by an equal amount of the acid extract of polished rice. The yeast was increased to 15 per cent on Dec. 4. There were two cases of leg weakness among this lot and both chicks died before the feeding trial terminated. The rate of growth of the remaining chicks was slow at first, but increased rapidly when additional vitamin B was supplied. The feathers were always in good condition. Three chicks, Nos. 2051, 2057, and 2060, continued on this diet until Jan. 19 when they were transferred to Ration 276. This mixture contained both rice fractions, but we do not regard it as superior to No. 231.

During the first four or five weeks growth was distinctly subnormal, and at the end of the seventh week five chicks were removed from the experiment. At the time we felt sure that this ration would not induce normal growth, and reduced the number in the group in order to lower feed costs. The graph however, Fig. 23, shows distinctly that the rate of growth was increasing rapidly at this time, and all three of the remaining birds ultimately attained weights normal for their age. At the

end of the eleventh week these three specimens were placed on Ration 276, which contained both of the polished rice fractions. We do not believe, however, that the change improved the ration. Our present position is that whatever the active material in polished rice may be, it is soluble in dilute acetic acid.

It was evident that Ration 231, containing 1 per cent rice extract, was markedly superior to our basal ration for that series, and the growth promoting properties of a small amount of this adjuvant had been very surprising. To substantiate our results the feeding trial was repeated, with a slight change in the ration, beginning Feb. 24, 1925. This lot began with Ration 294, but it was necessary to make a few changes in the diet during the feeding trial. It is to be noted that this ration was very similar to the basal ration for this series (Fig. 9), differing only in that one per cent of starch was replaced by the rice extract. Vitamin B was supplied daily in tablet form, and each chick received one tablet daily for the first eighteen days. At this time the supply of vitamin tablets had been exhausted and 15 per cent of dried yeast was incorporated in the ration, replacing an equal amount of starch. This ration was fed

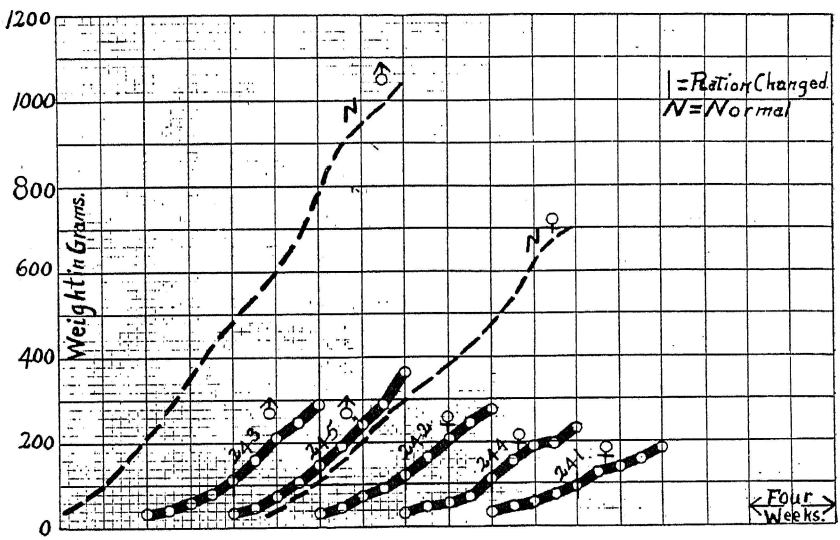

Fig. 24.—This group received a ration, No. 294, composed of washed casein 20, Crisco 10, cod liver oil 5, salts (14 A) 4, rice extract 1, cellulose 3, and starch 57. Vitamin B was supplied in the form of Harris-Vitamin tablets. On the twenty-sixth day the diet was changed to Ration 311, in order to supply an adequate amount of vitamin B, and to note the effect of a salt mixture containing a silicate upon the condition of the feathers. The change of ration consisted in replacing 6 per cent of starch with an equal weight of yeast, and replacing the salts with an equal weight of salt mixture 14 B. The rate of growth of this lot was slightly subnormal, but considerably better than that of the basal ration for this series (Fig. 9). This again indicates that the growth promoting factor or factors carried by polished rice can be extracted by dilute acetic acid.

until March 24, when the diet was changed to Ration 311, and in addition each chick was given 100 mgm. vitamin concentrate daily. Ration 311 also contained 6 per cent dried yeast, that we might be more completely assured of its adequacy in vitamin B. We also considered the possibility that the chick may require silica, so we changed from salt mixture 14 A to 14 B, which contained 0.5 per cent of that substance. Although the rate of growth of this lot was not entirely normal (Fig. 24), there were no symptoms of leg weakness. The chicks of this lot were feathered normally, which seemed to be characteristic of those receiving a rice ration.

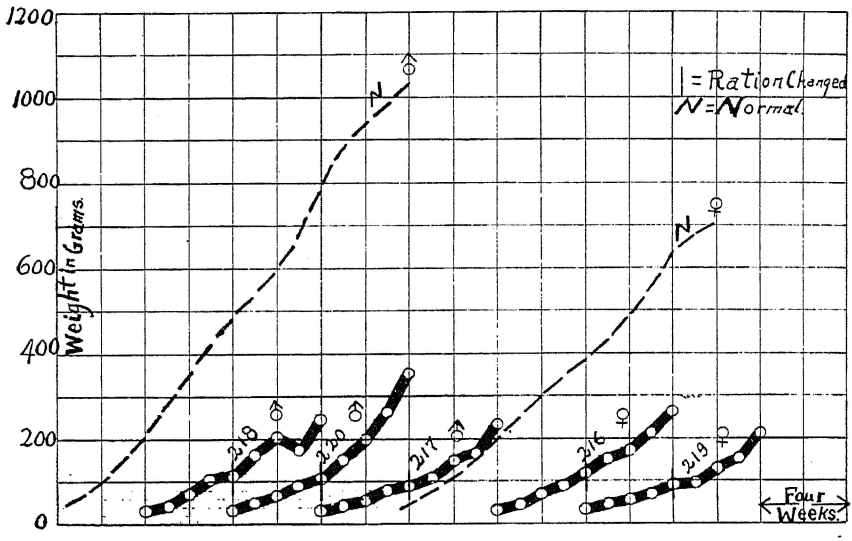

Fig. 25.—This group received Ration 291, which was composed of washed casein 16, Crisco 10, cod liver oil 5, salts (14 A) 4, rice extract 1, cellulose 3, and washed rice 61. Each chick received one vitamin tablet daily as a source of vitamin B. On the twenty-fourth day the diet was changed to Ration 308, which contained 6 per cent yeast and salt mixture 14 B. In addition each chick received two vitamin tablets daily. There were no symptoms of leg weakness among the lot, and the rate of growth was practically normal. There is no evidence that the slight alterations in the ration had any effect on the rate of growth. The feathers appeared normal, which seems to be characteristic of chicks grown on rice rations. In a comparison with Ration 294, it does not appear that this ration was improved to any extent by the substitution of washed rice for corn starch.

To determine the effect of our process of fractionating upon the nutritive value of polished rice, a lot of five chicks was placed on Ration 291 on Feb. 24. This ration was similar to the one just described, but contained both rice fractions. Again it was necessary to make changes in the ration, until finally they were receiving Ration 308. Each chick also received two vitamin tablets daily. There is no evidence that the various alterations in the diet affected the rate of growth in any way.

At the end of eight weeks the feeding trial terminated, and all of the chicks were in a good state of nutrition at this time. There were no symptoms of leg weakness among the lot, but the rate of growth was not quite normal. In all other respects however, the chicks were all that could be desired. The history and the rate of growth of this lot is almost identical with the lot receiving the rice extract (Fig. 27). The results do not indicate that the washed rice incorporated in such a ration was much superior, from a nutritional standpoint, to an equal weight of corn starch.

After a former ration, containing 15 per cent of dried ox liver, had proven completely successful in every respect (Fig. 13) we incorporated the various fractions of this natural food stuff in our diets. On Feb. 24, 1925, three lots of five chicks each were placed on diets containing these fractions.

One lot received Ration 188, which contained the liver protein. In addition each chick received one vitamin tablet daily as the source of vitamin B. In order to insure an adequate supply of vitamin B the diet was changed to Ration 305 on March 24. The nature of the changes is the same as described in a preceding paragraph (Page 29). There were no cases of leg weakness among this lot and all chicks were alive at the end of eight weeks when the experiment terminated.

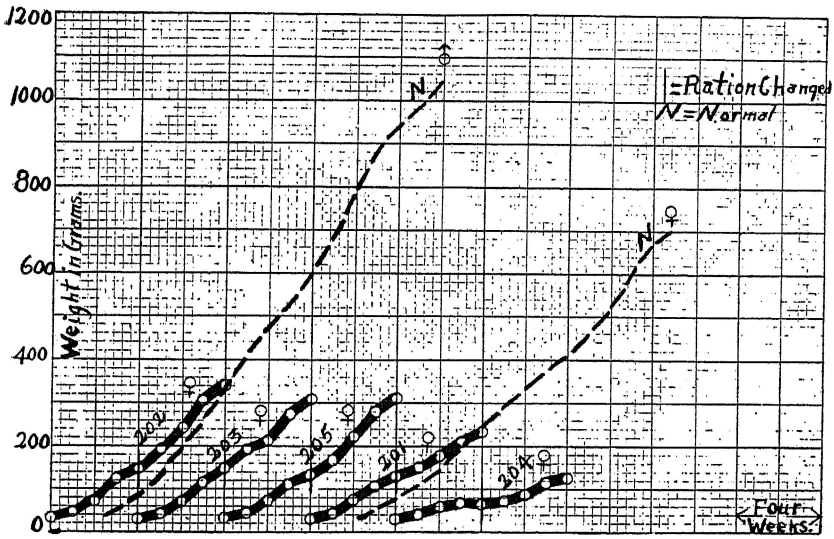

Fig. 26.—This group began with Ration 288, which was composed of liver protein 9.5, washed casein 10.5, Crisco 10, salts 4, cod liver oil 5, cellulose 3, and starch 58. Vitamin B tablets were supplied daily. On the twenty-sixth day, 6 per cent of starch was replaced by an equal weight of dried yeast. The rate of growth among this lot was slightly below normal, but it was considerably better than that on the basal ration for this series (Fig. 9). It appears that our basal ration was improved materially by the substitution of liver protein for an equal weight of casein.

The rate of growth was subnormal (Fig. 26), but considerably better than that of the group on the basal ration for this series (Fig. 9).

The second lot received Ration 289, which contained the ether and alcohol extracts obtained in preparing the liver protein, used in Ration 288. In addition, each chick received one to three tablets of the vitamin concentrate daily as the source of vitamin B, until the eighteenth day when the supply was exhausted. At this time 15 per cent of the starch was replaced by yeast. Leg weakness appeared among this lot on March 9, and later three chicks died with this disease. On March 24, the diet was changed to Ration 306, which contained 6 per cent of yeast, and the

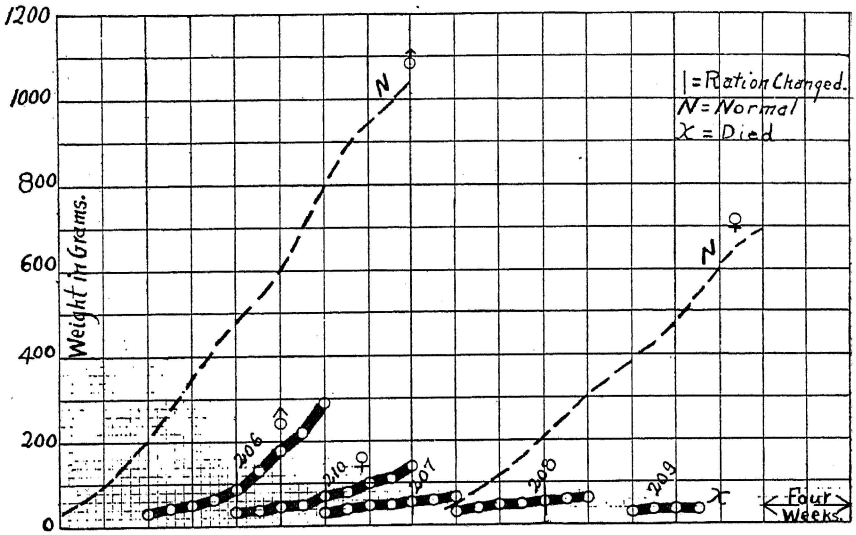

Fig. 27.—This lot was first fed Ration 289, composed of washed casein 20, ether extract of liver 1.8, alcohol extract of liver 3.7, Crisco 8.2, cod liver oil 5, salts 4, cellulose 3, and starch 54.3. Vitamin B tablets in addition were supplied daily. A number of cases of leg weakness appeared among the lot, and 6 per cent of the starch was replaced with an equal weight of dried yeast on the twenty-sixth day. Three of the chicks did not recover from leg weakness and the rate of growth of the remaining two was very satisfactory, but very similar to those on the basal ration. Evidently the ration was not improved by the substitution of the liver extracts.

salts were replaced by an equal amount of salt mixture No. 14 B. In addition each chick received 100 mgm. vitamin concentrate daily. The rate of growth of this group was very unsatisfactory and only two chicks were alive at the end of the eighth week. These had the ruffled feathers which are so characteristic of our synthetic rations. The results (Fig. 27) do not indicate that this ration was improved by the substitution of the liver extracts.

The third lot (Fig. 28) received Ration 290, composed of casein 10.5, liver protein 9.5, liver ether extract 1.8, liver alcohol extract 3.7,

Crisco 8.2, cod liver oil 5, cellulose 3, salts (14 A) 4, and starch 54.3. Each chick received one tablet daily as the source of vitamin B. It is to be noted that this ration contained the various liver fractions in the same proportions as they existed in the liver itself, or an amount equal to that incorporated in a former ration, No. 230 (Fig. 13). For the reasons already given various changes were made in the ration, and on March 24, the diet was changed to Ration 307. This change of ration consisted in replacing 6 per cent of starch with an equal amount of yeast, and replacing the salt mixtures by an equal amount of salt mixture No. 14-B. All five chicks survived the period of the feeding trial, and two grew at

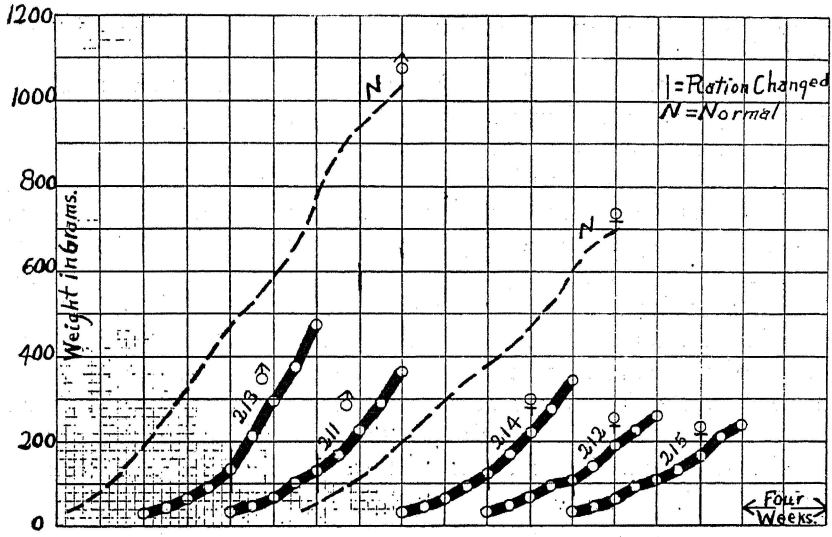

Fig. 28.—This group received Ration 290, which contained both of the dried liver fractions used in the two prevoius lots. It is to be noted that the amount of the fractions incorporated in the ration was equivalent to 15 per cent of the dried unextracted liver. Growth among this lot was fairly satisfactory, but not as much so as with a former lot receiving Ration 230, Fig. 13. No cases of leg weakness were observed among this group, but only two chicks attained a normal rate of growth. The results indicate that the nutritional properties of the dried liver has at least been slightly affected by the procedure of fractionating.

practically a normal rate (Fig. 28). There were no symptoms of leg weakness, but the general history of this group was less satisfactory than that of the lot receiving Ration 230, containing an equivalent amount of the dried liver. Evidently the growth promoting factor had been affected in the process of extraction.

DISCUSSION AND SUMMARY

We have successfully reared a considerable number of chicks on synthetic diets, and in most attempts the mortality has been comparatively low. There has been considerable variability among the individual chicks, and not infrequently we have observed unexpected nutrtional disaster, usually identified as "leg weakness." We have only one explanation to offer as to the possible cause of such a nutritional disturbance, and that is, the diet was deficient in vitamin B. We have treated a number of chicks, affected with this disease, with rich sources of vitamin B and a cure resulted. However, if the treatment is not begun in an early stage of the disease, the chick will either die or lose control of its legs permanently, although it may continue to grow and appear normal in other respects.

The exposure of chicks receiving our synthetic rations to direct sunlight (short periods) was of no apparent benefit.

At first our results indicated that cellulose was superior to agar as a source of roughage, but later experiments do not confirm this view, and we are of the opinion that the value of agar and cellulose as a source of roughage is approximately equal. There is an indication that chicks receiving the agar ration require slightly more vitamin B than those receiving the cellulose ration. We are at a loss to explain this variation.

Chicks receiving these synthetic rations developed a ruffled condition of the feathers which seems to be characteristic for the diet, however as the chicks approached maturity the feathers became more perfect, and the birds ultimately seemed entirely normal..

Meat protein substituted for part of the casein did not improve the diet.

When natural food stuffs such as dried egg yolk, polished rice, and dried liver were incorporated in the diet, growth was markedly acceleated and practically the optimum rate was attained. These observations were confirmed under slightly different conditions, by other feeding trials. Only a few cases of "leg weakness" have been observed among chicks receiving such rations. Chicks receiving these rations containing the natural food stuffs developed a good coat of feathers and appeared normal in all respects. This is especially true of the lots receiving the polished rice.

As to the successful feeding of egg yolk and dried liver, there is a multiplicity of possible explanations as these substances are of a more or less complex structure. We are of the opinion, however, that at least part of the success can be attributed to the increase in the vitamin B content of the diet. The more favorable outcome following the use of polished rice is unexpected from any point of view. This substance con-

tains starch, a low percentage of protein, though it is of good biological value, an insignificant quantity of mineral elements, and from a quantitative standpoint practically nothing less.

In our attempts, by fractionating, to localize the growth promoting factor carried by these natural food stuffs, we have been for the most part unsuccessful, but not completely so. The various fractions of egg yolk did not make our diets complete when fed separately, however, when they were combined in the same ratio, the resulting rate of growth was practically the same as when the diet contained the original dried egg yolk. This would indicate that the active factor was not affected materially by the process of fractionating.

When the liver extracts were incorporated in our ration, it did not appear to be improved in any way. However when the protein fraction was included in the ration a marked acceleration of growth resulted, but the rate was slightly subnormal. From this, it seems logical to infer that at least part of the active factor remains in the protein. When the two fractions were combined in the ration in the proper ratio to form 15 per cent dried liver, the resulting rate of growth was approximately normal, but less satisfactory than in the lot receiving the original dried liver. It appears that our process of extraction had affected its nutritional properties. The chicks receiving the diets containing the fractions of egg yolk and dried liver have shown the typical ruffled condition of the feathers.

Chicks receiving diets containing the extracted polished rice, grew at a subnormal rate, but may have had slightly smoother feathers than those on the basal ration. However the chicks receiving the diet containing one per cent of the extracted material grew at approximately a normal rate. These chicks were well feathered and appeared normal in all respects. This is also true when the ration contained both fractions. It seems evident that the growth promoting factor carried by the polished rice is removed by extracting with dilute acetic acid.

We believe that the results obtained throughout this investigation have emphasized the importance of an adequate supply of vitamin B in the diet. The requirement of chicks for this vitamin is high. However our data do not indicate that this is the only factor which has played an important part in determining the success or failure of our ration for nutritional purposes. It will be recalled that when additional vitamin B was offered chicks, receiving the basal diet, the rate of growth was not improved.

It is useless to speculate on the subject, but we are acting on the hypothesis that the nutritional requirements of chicks differ ma kedly in at least one respect from the known requirements of mammals.

Evidence supporting such a supposition is no more than circumstantial and we are acting on this theory partly because so far as we are aware there is no record of baby chicks attaining a normal growth rate when consuming synthetic diets. In this connection one naturally thinks of palatability, but from our point of view such an explanation offers a name for the difficulty and offers no real explanation. A ration may be unpalatable merely because it is at least partially inadequate. To our minds the favorable outcome of feeding trials with ground polished rice substituted for the corn starch of our synthetic diets supports this view.

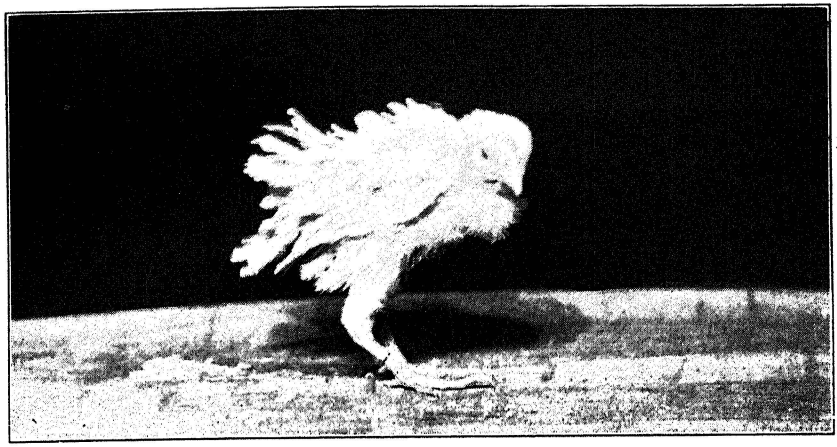

Fig. 29.—Chick 2001 (from Fig. 6), age 41 days. The ruffled condition of the feathers characteristic of chicks receiving synthetic diets, is apparent.

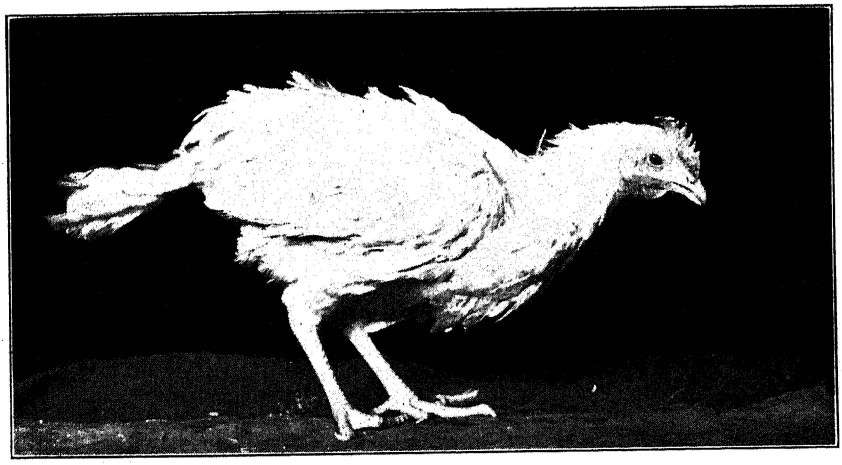

Fig. 30.—Chick 1907 (from Fig. 1), age, 101 days. This chick received the same type of diet as the one in Fig. 29. The increased rate of growth and improvement in the condition of the feathers, as the chick approaches maturity is evident.

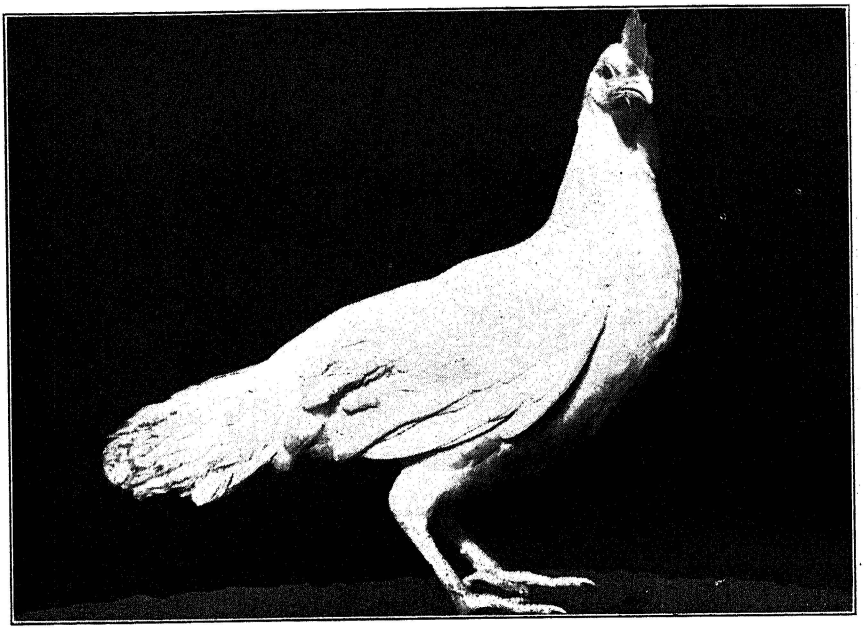

Fig. 31.—Chick 3778, not previously described. Age, 203 days. This chick received a synthetic diet, and showed the characteristic condition of the feathers during growth. At this age, however, the bird appeared normal in every respect.

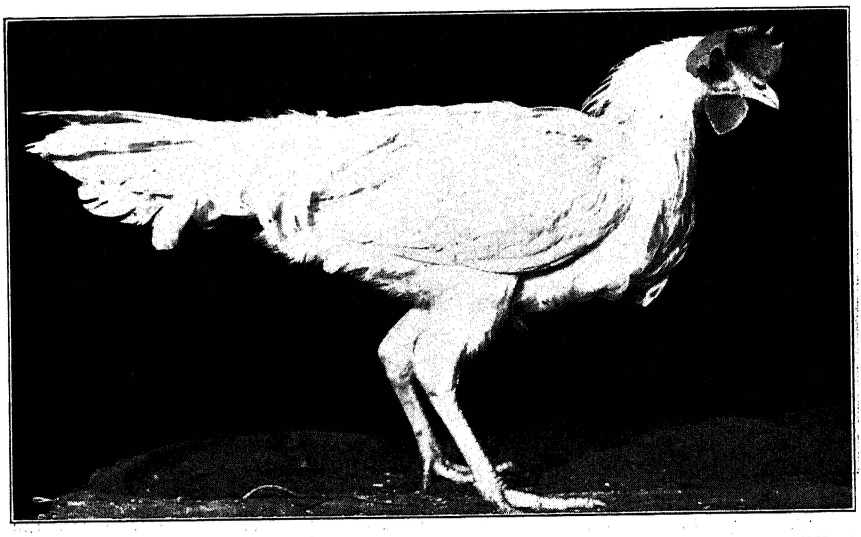

Fig. 32.—Chick 1937 (Fig. 11), age 101 days. The ration included polished rice. The performance of this group was very satisfactory. With one exception the rate of growth was normal, and the feathers were always in a perfect condition.

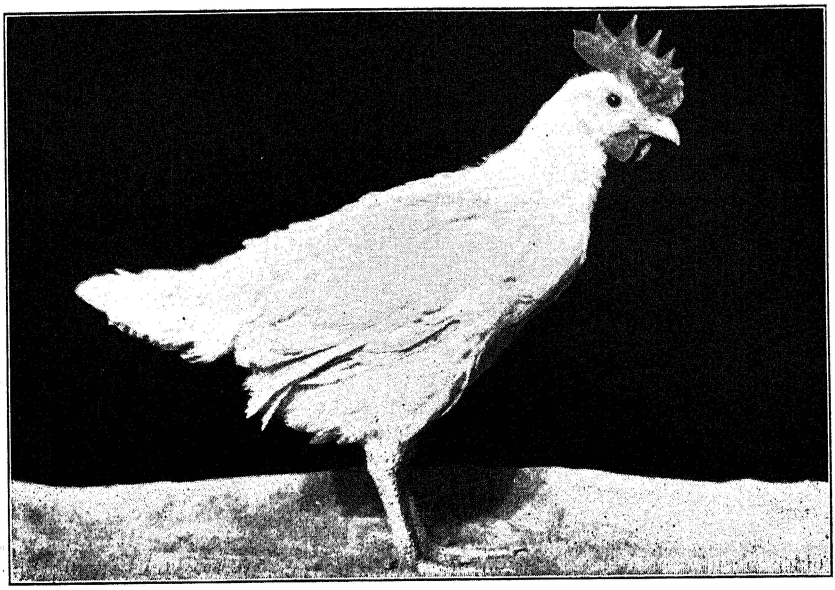

Fig. 33.—Chick 1952 (from Fig. 12), age 40 days. This chick received a ration containing egg yolk. It grew rapidly, but all of the group had a rough coat of feathers.

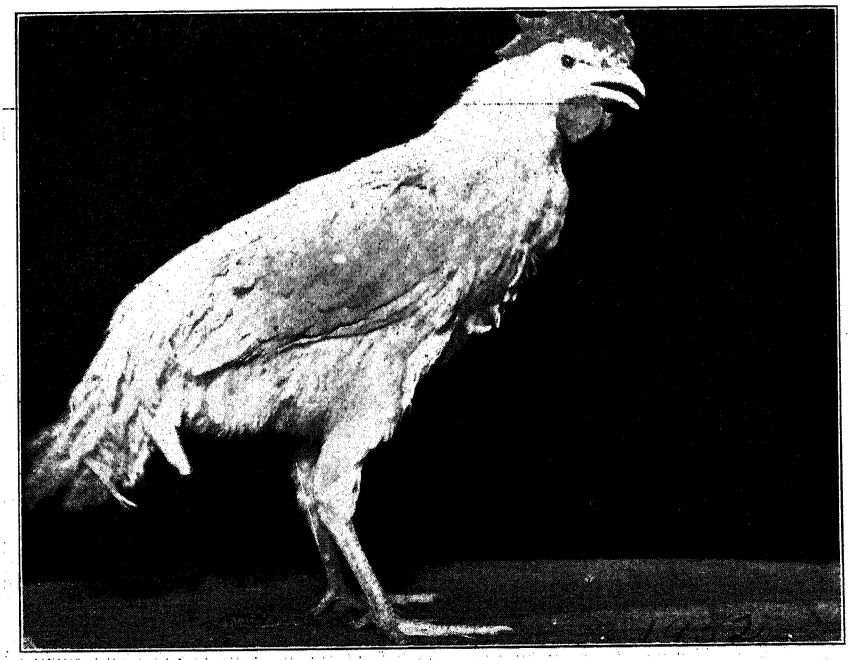

Fig. 34.—Chick 1952 (from Fig. 12), age 101 days. This is the same chick as in Fig. 33, but at a later date. The condition of the feathers has improved.

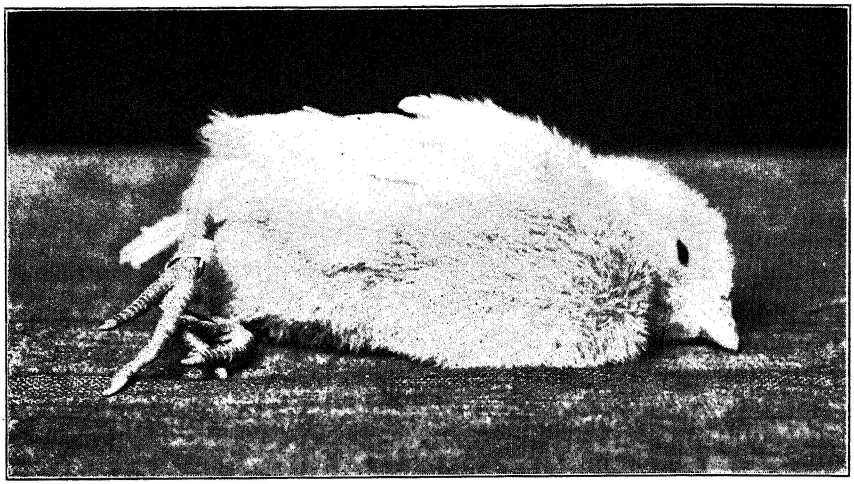

Fig. 35.—Chick 2015 (from Fig. 20), age 16 days. This chick has a severe attack of leg weakness. We observed similar cases very frequently during this investigation.

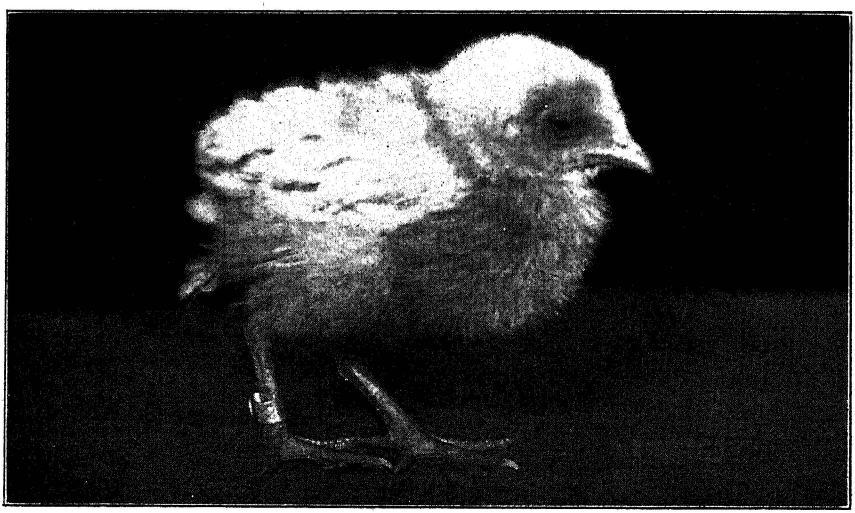

Fig. 36.—Chick 2015 (from Fig. 20), age 17 days. This is the same chick as shown above, one day after it had been treated with yeast emulsion. At this time it had apparently recovered.

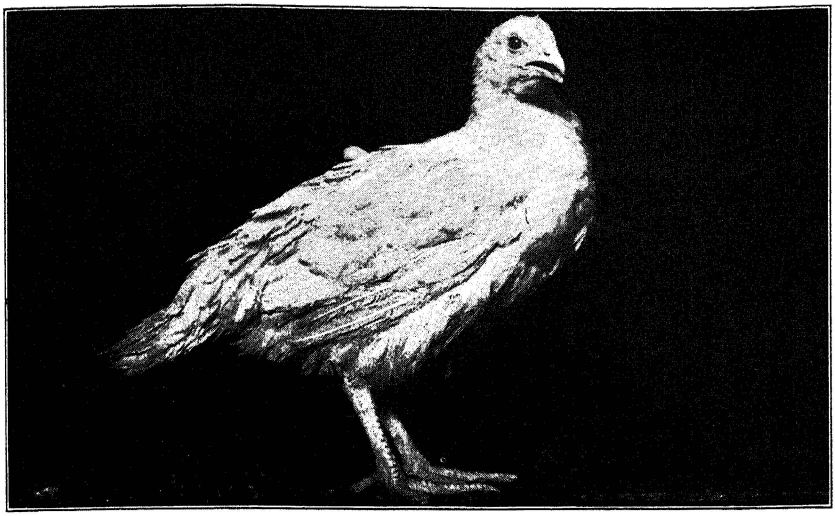

Fig. 37.—Chick 2015 (from Fig. 20), age 101 days. This is another photograph of the chick shown in Fig. 36, taken at a later date. At this time the chick appeared to be in perfect physical condition.

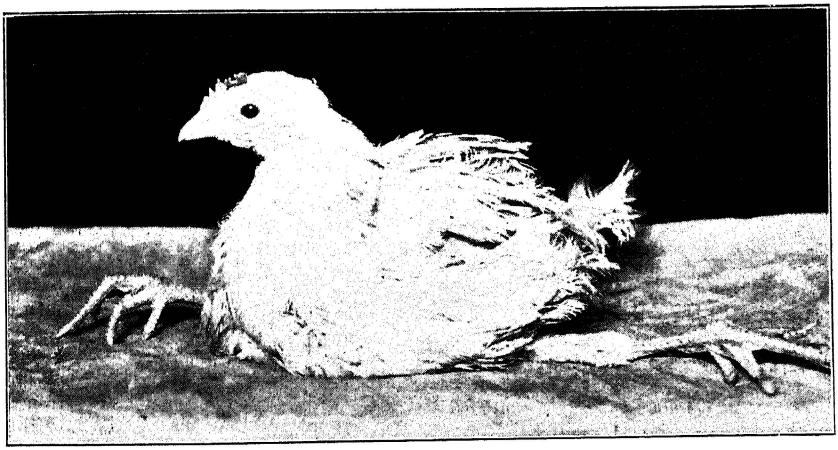

Fig. 38.—Chick 1978 (from Fig. 17), age 96 days. A condition of tonic spasticity is always characteristic of leg weakness in the cases of polyneuritis we have observed. When the symptoms develop suddenly and the feeding of additional yeast or yeast extract is begun promptly, recovery is usually complete, or nearly so. If treatment is sufficiently delayed however we find that changes in the ration are ineffective so far as the paralysis is concerned, though the general well-being may be vastly improved.

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APPENDIX

Composition of Rations

Figs. 1 and 2*

Number	167[1]	241
Casein	20.0	20.0
Starch	52.0	50.0
Yeast	6.0	8.0
Crisco	10.0	10.0
Cod Liver Oil	5.0	5.0
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0

[1]This ration was fed until July 28, 1924.

Fig. 3

Number	168[2]	242
Casein	20.0	20.0
Starch	52.0	50.0
Crisco	10.0	10.0
Yeast	6.0	8.0
Cod Liver Oil	5.0	5.0
Salt Mixture	4.0	4.0
Agar	3.0	3.0

[2]This ration was fed until July 28, 1924.

Fig. 4

Number	241[1]	223[2]	259
Casein	20.0	20.0	16.0
Starch	50.0	48.0	0.0
Polished Rice	0.0	0.0	52.0
Yeast	8.0	10.0	10.0
Crisco	10.0	10.0	10.0
Cod Liver Oil	5.0	5.0	5.0
Salt Mixture	4.0	4.0	4.0
Cellulose	3.0	3.0	3.0

[1]This ration was fed until October 1, 1924.
[2]This ration was fed until December 10, 1924.

Fig. 5

Number	247[1]	221[2]	257
Casein	15.0	15.0	0.0
Meat Protein	5.0	5.0	20.0
Starch	50.0	48.0	48.0
Yeast	8.0	10.0	10.0
Crisco	10.0	10.0	10.0
Cod Liver Oil	5.0	5.0	5.0
Salt Mixture	4.0	4.0	4.0
Cellulose	3.0	3.0	3.0

[1]This ration was fed until October 1, 1924.
[2]This ration was fed until December 10, 1924.

*These numbers refer to the charts in the text, that indicate the growth rate of chicks on the ration described.

Fig. 6

Number	226[1]	249
Washed Casein	20.0	20.0
Starch	53.0	48.0
Yeast	10.0	15.0
Crisco	10.0	10.0
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0
Unsaponified residue equivalent to 5% Cod Liver Oil.		

[1]This ration was fed until December 4, 1924.

Fig. 7

Number	232[1]	254
Washed Casein	20.0	20.0
Starch	53.0	48.0
Yeast	10.0	15.0
Crisco	10.0	10.0
Salt Mixture	4.0	4.0
Agar	3.0	3.0
Unsaponified residue equivalent to 5% Cod Liver Oil.		

[1]This ration was fed until December 4, 1924.

Fig. 8

Number	292[1]	309
Washed Casein	20.0	20.0
Crisco	10.0	10.0
Cod Liver Oil	5.0	5.0
Salt Mixture (14 A)	4.0 (14 B)	4.0
Agar	3.0	3.0
Starch	58.0	52.0
Vitamin Tablets daily	1-3	2
Yeast	0.0	6.0

[1]This ration was fed until March 16, 1925. At that time the supply of tablets was exhausted and 15 per cent of yeast was incorporated in the ration as the source of vitamin B. On March 24, the diet was changed to Ration 309.

Fig. 9

Number	293[1]	310
Washed Casein	20.0	20.0
Crisco	10.0	10.0
Cod Liver Oil	5.0	5.0
Salt Mixture (14 A)	4.0 (14 B)	4.0
Cellulose	3.0	3.0
Starch	58.0	52.0
Vitamin Tablets daily	1-3	2
Yeast	0.0	6.0

[1]This ration was fed until March 24. On March 16, the supply of tablets was exhausted and 15 per cent of yeast was incorporated in the ration as the source of vitamin B. On March 24, the diet was changed to Ration 310.

Fig. 10

Number	195[1]	244
Casein	16.8	16.8
Starch	52.0	50.0
Yeast	6.0	8.0
Crisco	3.2	3.2
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0
Cod Liver Oil	5.0	5.0
Dried Egg Yolk	10.0	10.0

[1]This ration was fed until July 28, 1924.

Fig. 11

Number	237[1]	240
Casein	16.0	16.0
Polished Rice	56.0	54.0
Crisco	10.0	10.0
Yeast	6.0	8.0
Cod Liver Oil	5.0	5.0
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0

[1]This ration was fed until July 28, 1924.

Fig. 12

Number	244[1]	217[2]	223
Casein	16.8	16.8	20.0
Starch	50.0	48.0	48.0
Yeast	8.0	10.0	10.0
Crisco	3.2	3.2	10.0
Cod Liver Oil	5.0	5.0	5.0
Salt Mixture	4.0	4.0	4.0
Cellulose	3.0	3.0	3.0
Dried Egg Yolk	10.0	10.0	0.0

[1]This ration was fed until October 1, 1924.
[2]This ration was fed until December 1, 1924.

Fig. 13

Number	230
Dried Liver	15.0
Meat Protein	6.0
Starch	47.0
Yeast	10.0
Crisco	10.0
Salt Mixture	4.0
Cod Liver Oil	5.0
Cellulose	3.0

Fig. 14

Number	196[1]	245
Casein	16.8	16.8
Starch	52.0	50.0
Yeast	6.0	8.0
Crisco	10.0	10.0
Salt Mixture	4.0	4.0
Cod Liver Oil	5.0	5.0
Cellulose	3.0	3.0
Yolk Protein	3.2	3.2

[1]This ration was fed until July 28, 1924.

Fig. 15

Number	197[1]	246
Casein	20.0	20.0
Starch	52.0	50.0
Yeast	6.0	8.0
Crisco	3.2	3.2
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0
Cod Liver Oil	5.0	5.0
Yolk Lipins	6.8	6.8

[1]This ration was fed until July 28, 1924.

Fig. 16

Number	245[1]	218[2]	255
Casein	16.8	16.8	16.8
Starch	50.0	48.0	48.0
Yeast	8.0	10.0	10.0
Crisco	10.0	10.0	3.2
Cod Liver Oil	5.0	5.0	5.0
Salt Mixture	4.0	4.0	4.0
Cellulose	3.0	3.0	3.0
Yolk Protein	3.2	3.2	3.2
Liver Lipins	0.0	0.0	6.8

[1]This ration was fed until October 1, 1924.
[2]This ration was fed until December 10, 1924.

Fig. 17

Number	246[1]	219[2]	256
Casein	20.0	20.0	16.8
Starch	50.0	48.0	48.0
Yeast	8.0	10.0	10.0
Salt Mixture	4.0	4.0	4.0
Crisco	3.2	3.2	3.2
Cellulose	3.0	3.0	3.0
Cod Liver Oil	5.0	5.0	5.0
Yolk Lipins	6.8	6.8	6.8
Liver Protein	0.0	0.0	3.2

[1]This ration was fed until Oct. 1, 1924.
[2]This ration was fed until December 10, 1924.

Fig. 18

Number	243[1]	220[2]	223
Casein	16.8	16.8	20.0
Starch	50.0	48.0	48.0
Yeast	8.0	10.0	10.0
Crisco	3.2	3.2	10.0
Salt Mixture	4.0	4.0	4.0
Cod Liver Oil	5.0	5.0	5.0
Cellulose	3.0	3.0	3.0
Yolk Protein	3.2	3.2	0.0
Yolk Lipins	6.8	6.8	0.0

[1]This ration was fed until October 1, 1924.
[2]This ration was fed until Dec. 10, 1924.

Fig. 19

Number	248[1]	222[2]	258
Casein	16.0	16.0	16.0
Polished Rice (Extracted)	52.0	52.0	51.0
Yeast	10.0	10.0	10.0
Crisco	10.0	10.0	10.0
Salt Mixture	4.0	4.0	4.0
Cod Liver Oil	5.0	5.0	5.0
Cellulose	3.0	3.0	3.0
Rice Extract	0.0	0.0	1.0

[1]This ration was fed until October 1, 1924.
[2]This ration was fed until December 10, 1924.

Fig 20

Number	227[1]	250
Washed Casein	16.8	16.8
Starch	48.0	43.0
Crisco	3.2	3.2
Yeast	10.0	15.0
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0
Meat Protein	3.2	3.2
Cod Liver Oil	5.0	5.0
Yolk Lipins	6.8	6.8

[1]This ration was fed until December 4, 1924.

Fig. 21

Number	228[1]	251
Washed Casein	16.8	16.8
Starch	48.0	43.0
Yeast	10.0	15.0
Crisco	3.2	3.2
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0
Cod Liver Oil	5.0	5.0
Butter Fat	6.8	6.8
Yolk Protein	3.2	3.2

[1]This ration was fed until December 4, 1924.

Fig. 22

Number	229[1]	252
Washed Casein	16.8	16.8
Starch	48.0	43.0
Yeast	10.0	15.0
Crisco	3.2	3.2
Salt Mixture	4.0	4.0
Cellulose	3.0	3.0
Cod Liver Oil	5.0	5.0
Butter Fat	6.8	6.8
Meat Protein	3.2	3.2

[1]This ration was fed until December 4, 1924.

Fig. 23

Number	231[1]	253[2]	276
Washed Casein	20.0	20.0	16.0
Starch	47.0	42.0	0.0
Extracted Rice	0.0	0.0	51.0
Yeast	10.0	15.0	10.0
Crisco	10.0	10.0	10.0
Salt Mixture	4.0	4.0	4.0
Cod Liver Oil	5.0	5.0	5.0
Cellulose	3.0	3.0	3.0
Rice Extract	1.0	1.0	1.0

[1]This ration was fed until December 4, 1924.
[2]This ration was fed until January 19, 1925.

Fig. 24

Number	294[1]	311
Washed Casein	20.0	20.0
Crisco	10.0	10.0
Cod Liver Oil	5.0	5.0
Salt Mixture	(14 A) 4.0	(14 B) 4.0
Cellulose	3.0	3.0
Rice Extract	1.0	1.0
Starch	57.0	51.0
Vitamin Tablets Daily	1	2
Yeast	0.0	6.0

[1]This ration was fed until March 16, 1925. At that time the supply of tablets was exhausted and 15 per cent of yeast was incorporated in the ration as the source of vitamin B. On March 24, the diet was changed to Ration 311.

Fig. 25

Number	291[1]	308
Washed Casein	16.0	16.0
Crisco	10.0	10.0
Cod Liver Oil	5.0	5.0
Salt Mixture	(14 A) 4.0	(14 B) 4.0
Cellulose	3.0	3.0
Rice Extract	1.0	1.0
Washed Rice	61.0	55.0
Vitamin Tablets Daily	1	2
Yeast	0.0	6.0

[1]This ration was fed until March 16, 1925. At that time the supply of tablets was exhausted and 15 per cent of yeast was incorporated in the ration as the source of vitamin B. On March 24 the diet was changed to Ration 308.

Fig. 26

Number	288[1]	305
Liver Protein	9.5	9.5
Washed Casein	10.5	10.5
Crisco	10.0	10.0
Salt Mixture (14 A)	4.0	(14 B) 4.0
Cod Liver Oil	5.0	5.0
Cellulose	3.0	3.0
Starch	53.0	52.0
Vitamin Tablets Daily	1	2
Yeast	0.0	6.0

[1]This ration was fed until March 16, 1925. At that time the supply of tablets was exhausted and 15 per cent of yaest was incorporated in the ration as the source of vitamin B. On March 24, the diet was changed to Ration 305.

Fig. 27

Number	289[1]	306
Washed Casein	20.0	20.0
Liver Ether Extract	1.8	1.8
Liver Alcohol Extract	3.7	3.7
Crisco	8.2	8.2
Cod Liver Oil	5.0	5.0
Salt Mixture (14 A)	4.0	(14 B) 4.0
Cellulose	3.0	3.0
Starch	54.3	48.3
Vitamin Tablets Daily	1-3	4
Yeast	0.0	6.0

[1]This ration was fed until March 16, 1925. At that time the supply of tablets was exhausted and 15 per cent of yeast was incorporated in the ration as the source of vitamin B. On March 24, the diet was changed to Ration 305.

Fig. 28

Number	290[1]	307
Washed Casein	10.5	10.0
Liver Protein	9.5	9.5
Liver Ether Extract	1.8	1.8
Liver Alcohol Extract	3.7	3.7
Crisco	8.2	8.2
Cod Liver Oil	5.0	5.0
Cellulose	3.0	3.0
Salt Mixture (14 A)	4.0	(14 B) 4.0
Starch	54.3	48.3
Vitamin Tablets Daily	1	2
Yeast	0.0	6.0

[1]This ration was fed until March 16, 1925. At that time the supply of tablets was exhausted and 15 per cent of yeast was incorporated in the ration as the source of vitamin B. On March 24, the diet was changed to Ration 307.