# THE DIETARY FACTORS OPERATING IN THE PRODUCTION OF POLYNEURITIS.*

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Eykman,[1] Suzuki,[2] Funk,[3] and others have made it clear that continued feeding of a diet of polished rice induces polyneuritis in birds. Funk[4] has likewise shown that identical results come from feeding birds diets of highly purified foodstuffs. This pathological condition, he has shown, is relieved by the introduction into the diet of water or alcoholic extracts of several natural foodstuffs.

The studies of a number of investigators have now fully established the fact that animals cannot grow when limited to rations of carefully purified proteins, carbohydrates, fats, and salts.[5] McCollum and Davis[6] have shown that certain fats, as butter fat, egg yolk fat, kidney fat, and others contain something which greatly stimulates growth when added to a diet of casein, dextrin, lactose, and a salt mixture of appropriate composition. This substance is present in certain products of plant origin as well. Later they discovered that if the lactose was replaced by dextrin

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[1] Eykman, C., *Arch. path. Anat.*, 1897, cxlviii, 523; cxlix, 187; *Arch. Hyg.*, 1906, lviii, 150.

[2] Suzuki, U., Shimamura, T., and Odake, S., *Biochem. Z.*, 1912, xliii, 89.

[3] Funk, C., *Ergebn. Physiol.*, 1913, xiii, 125, gives an extensive résumé of the literature relating to the so called deficiency diseases, together with a complete bibliography of the older literature.

[4] Funk, *Z. physiol. Chem.*, 1914, lxxxix, 374.

[5] Hopkins, F. G., *J. Physiol.*, 1912, xliv, 425. Hopkins, F. G., and Neville, A., *Biochem. J.*, 1913, vii, 97. Funk, C., and Macallum, A. B., *Z. physiol. Chem.*, 1914, xcii, 13. Stepp, W., *Z. Biol.*, 1913, lxii, 405.

[6] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxi, 179.

in this ration the addition of butter fat did not induce growth. When this diet, containing butter fat, was supplemented with small additions of the water extract of boiled egg yolk, or water or alcoholic extracts of wheat germ, growth proceeded at the normal rate. They formulated the working hypothesis that in addition to the recognized essential constituents of a successful diet, *viz.*, protein, carbohydrate, fats, and inorganic salts, the growing animal requires two substances, or groups of substances; one, which is soluble in fats, is contained in fats from certain sources; the other, which is soluble in water and alcohol, is found widely distributed both in the animal and vegetable worlds.

Funk and Macallum[7] have pointed out that butter fat does not relieve polyneuritis in pigeons and also that with rations consisting, aside from the butter fat content, of carefully purified foodstuffs, does not induce growth in young rats and they clearly express their belief that "vitamines" are absent from butter fat.

By "vitamine" Funk and his coworkers indicate a class of substances the chemical nature of which is at present unknown. They are described as being precipitated by phosphotungstic acid and by mercuric chloride, but aside from this property there is no evidence that these substances of unknown constitution which are active in promoting growth, or in relieving the symptoms of polyneuritis contain an amino group. Furthermore, the prefix *vita* connotes an importance in biological processes paramount to that of certain other absolutely indispensable organic complexes, among which are a number of amino-acids. We feel that this term is not in harmony with a conservative tendency in the nomenclature of biological chemistry which should avoid the employment of a term which carries the idea that one indispensable complex is of greater importance biologically than another one equally indispensable. Furthermore, the evidence of the presence of an amino group in the substances under consideration is too slight to warrant the use of the ending *amine*, which carries with it a definite meaning in organic chemical nomenclature.

Hopkins[8] introduced the term "accessory" for the substances of unknown chemical character which are found in milk and which

[7] Funk and Macallum, *J. Biol. Chem.*, 1915, xxiii, 413.

[8] Hopkins, *J. Physiol.*, 1912, xliv, 425. Hopkins and Neville, *Biochem. J.*, 1913, vii, 97.

render the addition of small amounts of milk to rations consisting otherwise of purified food substances, so effective in promoting growth. The term accessory carries with it the idea of playing a subordinate rôle, and in this sense has been employed by writers on dietetics as synonymous with condiment. While we have, following Hopkins, employed this term up to the present time, we have felt that it is by no means satisfactory.

We would, therefore, suggest the desirability of discontinuing the use of the term vitamine, and the substitution of the term fat-soluble A and water-soluble B for the two classes of unknown substances concerned in inducing growth. These terms have the merit of not attributing extravagant values to these bodies, and they differentiate between the substances or groups of substances only with respect to their solubility relations, which is the only basis of differentiation at present known. These terms have the additional advantage that further letters may readily be introduced as investigation progresses, provided there proves to be more than a single representative of each class, and also that they will automatically fall into disuse when we come to possess definite knowledge of their chemical nature.

When McCollum and Davis[10] proposed the working hypothesis regarding the essential factors in the diet during growth which assumes the necessity of the two kinds of unknown substances mentioned above, they were fully aware of the apparent discrepancy between this hypothesis and the recorded experimental data which indicated that birds can be cured of polyneuritis by the employment of water extracts of certain natural foodstuffs. Such water extracts could scarcely be assumed to contain appreciable amounts of lipoidal material. In fact, nearly three years ago, one of us (M.) satisfied himself by experimental trial of the validity of the statement that a cure can be effected in this way.

In the present paper we wish to report an investigation of the relation to polyneuritis of the two classes of unknown dietary factors A and B as defined above.

[9] Howell, W. H., Textbook of Physiology, Philadelphia, 5th edition, 1913, 727, 905.

[10] McCollum and Davis, *J. Biol. Chem.*, 1915, xxiii, 231.

*Effects of Feeding Polished Rice with Butter Fat.*

Bird 792A was offered a ration of rice 95 and butter fat 5 per cent from Sept. 18 to 28. It steadily lost weight and was evidently not consuming an adequate amount of food. Beginning with Sept. 28 we forcibly fed the pigeon with 20 gm. daily of the rice and butter fat mixture. On Oct. 15 it manifested the first symptoms of polyneuritis. Oct. 16, it was in a very bad condition. It was given the alcoholic extract (95 per cent) of 10 gm. of wheat embryo. Oct. 17, the bird was in a nearly normal condition.

In all, nine birds were fed with this ration and the above protocol illustrates what may be anticipated as the result of confinement to this diet.

Our observations are in accord with those of Funk and Macallum indicating that butter fat does not protect against polyneuritis birds fed polished rice and the fat.

We early became convinced that pigeons cannot be depended upon to eat enough of such experimental rations as are described in this paper, so we adopted the practice of forced feeding twice daily. This was accomplished by inserting a tube into the crop and pouring the powdered ration into it. A glass rod served to discharge the food from the tube when necessary. About 10 gm. of food were administered at each feeding. Water was given twice daily in 10 cc. doses by means of a pipette.

*Effects of Feeding Polished Rice and Alcoholic Extracts of Fat-Free Wheat Embryo.*

500 gm. of wheat embryo were freed from fat by ether in a continuous extractor and the residue was then extracted during 18 hours with 95 per cent alcohol also in a continuous extractor of the Caldwell type. The alcoholic extract was evaporated on 200 gm. of dextrin (Preparation 1).

Bird 1113B was offered rice for voluntary consumption from Nov. 11 to 18. After this date the bird was forcibly fed with a mixture of casein 18, salts 3.7, dextrin 78.3 per cent.

This ration will be hereafter referred to as the casein ration. The composition of the salt mixture employed was as follows:

| | gm. | | gm. |
|---|---|---|---|
| NaCl | 0.173 | $CaH_4(PO_4)_2 . H_2O$ | 0.540 |
| $MgSO_4$ (anhydrous) | 0.266 | Ca lactate, $Ca(C_3H_5O_3)_2 . 5H_2O$ | 1.300 |
| $NaH_2(PO_4) . H_2O$ | 0.347 | Fe lactate Merck | 0.118 |
| $K_2HPO_4$ | 0.954 | | |

Funk[4] employed a ration of similar composition but containing butter fat, and observed that the birds were stricken wth polyneuritis after a period comparable to rice feeding.

Nov. 26, the pigeon was in the typical condition of severe polyneuritis. It was given Preparation 1 equivalent to 10 gm. of wheat embryo, morning and evening. Nov. 27, it was able to sit in a normal position, but could not use its legs. It was fed as on the 26th. Nov. 28, it was given 10 gm. of Preparation 1 and 10 gm. of the casein ration. Nov. 29, the bird could walk. We continued feeding as on Nov. 28 to Jan. 12 when the experiment was discontinued, the bird being in a normal condition.

The presence of the fat-soluble A in wheat embryo was established by McCollum and Davis.[6] They did not study the growth-promoting power of the isolated wheat embryo fat, however, and only after these experiments were well under way did we learn in another connection in feeding work with rats that the fat may be quantitatively removed from wheat embryo and yet the fat-free residue, when added to a ration of purified food materials, can induce in rats a growth curve entirely unlike those obtained with diets lacking the unknown A.[11] This led us to conclude that the recovery from polyneuritis and prolonged well-being of pigeons given alcoholic extract of fat-free wheat embryo, was not a demonstration of recovery and subsequent maintenance on a diet free from the fat-soluble A, since this is in the fat-free embryo and might be extracted with alcohol. We next sought to induce the recovery of pigeons by the administration of nearly lipoid-free preparations from other sources.

*Experiments with Potato Juice.*

Vedder and Clark[12] employed potato juice and found it inactive. Our experiments with potato juice gave results indicating that it possesses a moderate curative power.

Bird 1169B was forcibly fed from Nov. 14 to Dec. 4 with rice until polyneuritis was imminent. It was continued on rice and from Dec. 4 was given 12 cc. of fresh potato juice twice daily. The bird could walk very unsteadily from Dec. 8 to 18 when it so improved as to walk naturally, flew voluntarily to a perch, and appeared normal. The potato juice

[11] In a subsequent paper from this laboratory soon to appear this point will receive full consideration.

[12] Vedder, E. B., and Clark, E., *Philippine J. Sc., B.*, 1912, vii, 423.

was continued until Jan. 12 when the forced feeding was discontinued, the bird appearing normal. The ration of rice with potato juice evaporated upon it was offered for voluntary consumption. The consumption was entirely inadequate and the bird rapidly lost weight and strength during the first week. On changing it to a diet of corn meal it regained its normal condition.

Since the pigeons invariably grow emaciated when given diets which induce polyneuritis and may fully recover with proper dietary treatment without appreciable increase in weight, together with the fact that considerable fluctuation in body weight may result from the retention of food in the crop as the time for the onset of the pathological symptoms approaches, we do not believe that the value of our records is enhanced by the inclusion of the weight records. This portion of our data is therefore omitted.

*Experiments with Cabbage Juice.*

Bird 862B. This bird was forcibly fed with 25 gm. of polished rice per day from Nov. 11 to Dec. 6. From Nov. 24 the crop was continually packed with food and very hard. Locomotion was distinctly impaired. On Dec. 6 and thereafter two doses of cabbage juice of 15 cc. each were administered daily—morning and evening. The crop became soft when rice and cabbage juice were both administered and on Dec. 8 the bird walked much better. It never returned to a normal condition, however, and although the regular feeding with this mixture was continued, it occasionally regurgitated food and on Dec. 19 the crop was again packed with food. Dec. 20, it was in the typical polyneuritic condition. Three doses of cabbage juice were given on this date and the bird was still alive on the morning of the 21st and very weak, but the head was not retracted. Dec. 21, five doses of cabbage juice representing a total of 180 cc. evaporated to a pasty consistency were given. The symptoms of polyneuritis were in great measure relieved, but the bird was very weak and died at 9 a.m., Dec. 22. There is no doubt that the cabbage juice contains the curative factor, but apparently in a quite low concentration.

Bird 1113 B. Forcibly fed with rice from Jan. 12 to 29 when it showed by its retracted head and inability to walk that it was in the polyneuritic state. In the morning we gave it cabbage juice (15 cc.) and 10 gm. dextrin on which 70 cc. of cabbage juice had been evaporated. In the afternoon the same dosage was administered. Jan. 30, the bird was normal in its movements and appeared cured.

*Experiments with Oat Extract.*

Bird 1091H was forcibly fed polished rice from Nov. 12 to Dec. 6 when symptoms of polyneuritis appeared. It was fed Dec. 6, morning and

afternoon, 12 gm. of rice on which a water extract of 24 gm. of rolled oats had been evaporated. 500 gm. of finely ground rolled oats were shaken in a shaking machine with 2 liters of water for 1 hour. The material was then centrifuged and the supernatant liquid poured off and evaporated on 500 gm. of rice. At each feeding 12 cc. of water were introduced into the crop. This treatment was thereafter continued daily until Dec. 16. The recovery was slow, but progressive, the crop remaining hard. On Dec. 12 the crop was washed out with a stream of warm water introduced through a rubber tube. The bird was apparently normal on Dec. 16 and thereafter would fly up to a perch and remain there when not disturbed. Forced feeding of this ration was continued until Jan. 19. For 3 weeks preceding the termination of the experiment this bird was very spirited and would persistently attack and annoy any other bird which was introduced into its cage, so it had to be kept isolated.

This experiment is of special interest because of the behavior of rats when fed rolled oats alone as contrasted with a mixture of rolled oats plus butter fat. When confined to rolled oats or the oat kernel freed from hulls in the laboratory, young rats steadily lose weight. On adding butter fat (5 per cent) to the oats they slowly gain in body weight and continue to exhibit a well nourished appearance for months.[13] From this we draw the conclusion that the oat kernel contains little if any of the fat-soluble A, since without its addition even maintenance is not attained, while growth proceeds when this factor is added. Since a solvent such as water, which exerts a very slight solvent action on lipoidal material, removes from oats a substance which cures polyneuritis, it points strongly to the belief that the cure is affected without the intervention of the fat-soluble A. The possibility remains, of course, that the requirement of the fat-soluble A is small for maintenance as compared with the amount necessary for growth. Since both classes of these unknown essential components of the diet are present in the vegetable world, and we have as yet no method perfected for testing for very small quantities of either A or B, this point cannot yet be definitely decided. Since, however, a pigeon can be maintained in excellent condition, as was Bird 1091H, by a mixture of 20 gm. of rice plus the water extract of 24 gm. of rolled oats per day, and growth cannot be attained with the content of the fat-soluble A in 100 per cent of rolled oats, we feel safe in asserting that the maintenance requirement of the

[13] An extended study of the dietary deficiencies of the oat kernel is now in progress in this laboratory and will be reported in a later paper.

bird for the fat-soluble A is less than the growth requirement of the rat.

There appears to be good evidence that the fat-soluble A tends strongly to remain associated with non-lipoid materials. This we have mentioned above in connection with the growth-promoting power, with respect to this particular factor, which is exhibited by wheat embryo completely extracted with ether. Further support of this idea is found in the growth curves obtained by Osborne and Mendel[14] with their protein-free milk. These curves showing normal growth over periods of 3 months or more could have been obtained only with rations which provide this dietary factor. Since they have shown that ether extracts almost nothing from protein-free milk it follows that it is difficult to free food materials from this substance.

We have endeavored to advance in the matter of determining whether water extracts of lipoid-free foodstuffs can effect a cure of polyneuritis in pigeons. To this end 500 gm. of wheat embryo were extracted exhaustively with ether and afterwards with carbon tetrachloride. The extraction with carbon tetrachloride for $22\frac{1}{2}$ hours at a good rate in a continuous extractor removed only 0.07 per cent of material from the ether-extracted embryo. The residue after exhaustion with both these solvents was extracted with water by stirring the embryo into boiling water and adding acetic acid to facilitate the complete coagulation of dissolved protein. The material was centrifuged and the clear supernatant liquid was evaporated on 500 gm. of dextrin (Preparation 2). This product had curative properties in a high degree.

Bird 910A. Fed rice Dec. 21 to Jan. 10 when it manifested typical polyneuritis. Jan. 10, it was given two doses of 5 gm. each of the casein ration and of Preparation 2. Jan. 11, p.m., the bird was slightly improved, but was in danger of dying during the night, so it was given a dose of the curative substance prepared by extracting 10 gm. of Preparation 2 with water. Jan. 12, a.m., the bird was in a normal condition. Thereafter it was given the casein ration carrying the Preparation 2 equivalent to 5 per cent of lipoid-free germ. On Jan. 16 its crop was hard and only water was given in the morning. In the evening the crop was still packed and hard and the bird was again showing incipient polyneuritis so it was given the strong solution from 10 gm. of Preparation 2. Jan. 17, a.m., the bird was again

[14] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1912, xii, 81.

normal. Thereafter it was given daily 20 gm. of the casein ration carrying Preparation 2 equivalent to 10 per cent of lipoid-free embryo. It remained normal until Jan. 23, p.m., when it again had polyneuritis. It recovered again with the strong solution, and the experiment was discontinued.

This record was duplicated in all essentials with two other pigeons, and leaves no room to doubt that the water extract of wheat germ made lipoid-free by thorough extraction with ether and with carbon tetrachloride induces a complete recovery from polyneuritis.

*Experiments with Acetone Extract of Fat-Free Wheat Embryo.*

For the isolation of the curative agent it is of the utmost importance to have an extensive knowledge of the solubility of the substance in all the ordinary solvents. With a view to obtaining such evidence we have administered to polyneuritic pigeons the extract of wheat embryo prepared by first removing all ether-soluble matter, and then extracting the residue at a vigorous rate in an extractor of the Caldwell type with acetone for 18 hours. The acetone-soluble material from 500 gm. of wheat embryo, which amounted to 3.2 gm., was incorporated with 396.8 gm. of dextrin (Preparation 3).

Bird 1096. Ate polished rice voluntarily from Dec. 14 to Jan. 13, when it was in incipient polyneuritis. It was given water extract of 10 gm. of Preparation 3. Jan. 14 signs of paralysis had disappeared. Gave the same water extract and rice in the morning. In the afternoon gave water only, as the crop was still hard. Jan. 15, the bird was in good condition. Fed the casein ration carrying the acetone extract of 5 per cent of wheat germ. Jan. 18, the crop was hard so we gave water only. Jan. 19, the crop was empty and the usual food given. Jan. 20, the crop was rather hard, the bird walked unsteadily. It was given 10 gm. of food in the morning; only water in the afternoon as the crop was packed. Jan. 21, the crop was hard, the bird walked unsteadily. It was given the casein ration carrying 10 per cent of acetone extract of embryo, but otherwise like that fed after Jan. 15. Jan. 22 the bird had a severe attack of polyneuritis in the morning. We gave strong water extract of 10 gm. of Preparation 3. Repeated this at noon. In the afternoon it was still very ill, so it was given another dose at 4 o'clock. At 8 p.m. it was on its feet. The dose was repeated. Jan. 23, it was nearly normal. We gave it the water extract of 10 gm. of Preparation 3 plus 5 gm. casein ration. Jan. 24, the bird was in normal condition, and the experiment was discontinued.

The above protocol is typical of three records obtained with acetone extract of wheat embryo. The curative substance is extracted by this solvent, in the course of long continued extraction.

*Experiments with Benzene Extract of Fat-Free Wheat Embryo.*

1 kg. of wheat embryo was extracted for 18 hours with ether and the residue afterward extracted in a Caldwell extractor for 18 hours with benzene. The benzene was evaporated on 200 gm. of dextrin. The extract amounted to 3.6 gm. (Preparation 4).

Bird 1172 from Dec. 14 was fed the casein ration. On Jan. 19 it began to regurgitate its food. Jan. 23, it had a bad attack of polyneuritis and was given a water extract of 10 gm. of Preparation 4, morning and evening. Jan. 24, it was much better, but still had symptoms of the disease. The same treatment was repeated. Jan. 25, it was nearly normal. Gave it 10 gm. of Preparation 4 plus 10 gm. of the casein ration in the morning. In the afternoon it appeared normal.

Another bird was cured after a similar history by this preparation.

*Experiment with Ethyl Acetate Extract of Fat-Free Wheat Embryo.*

400 gm. of wheat germ were extracted with ether during 48 hours. The residue was extracted for 14 hours with ethyl acetate and the ethyl acetate evaporated on 400 gm. of dextrin (Preparation 5).

Bird 968B was forcibly fed with rice from Dec. 14 to Jan. 31 when it had a severe attack of polyneuritis. It was given a water extract of Preparation 5, equivalent to 10 gm. of wheat embryo, in the morning. In the afternoon of Jan. 31 it could walk fairly well, and the dose was repeated. Feb. 1, the bird was in a normal condition.

The results of these experiments help in clarifying the apparent lack of harmony in the dietary requirements of the mammal during growth, and the relief of polyneuritis in birds. Both the fat-soluble A and the water-soluble B must be present in the diet of the mammal during growth. The absence of the former leads to failure to grow, to emaciation, and to a pronounced inflammation of the eyes, all of which conditions, provided the other factors in

the ration are adequate,[15] are promptly relieved by the administration of fats of the group which possess the peculiar properties of butter fat. No matter how generous may be the supply of the fat-soluble A, failure of nutrition will result, provided an adequate amount of the water-soluble B is not also furnished by the diet.[16] Judging from the appearance of serious nutritional disturbances ending in death, which result from a shortage of the fat-soluble A, and the emaciation, weakness, and death which follow restriction to a diet inadequate in its content of the water-soluble B, *it seems certain that both these classes of unknown dietary constituents are essential for maintenance as well as for growth.*

## SUMMARY OF CONCLUSIONS.

1. The data presented in this paper show conclusively, we believe, that in the production of polyneuritis in birds by exclusive rice feeding or exclusive feeding of a ration made up of purified foodstuffs, the degeneration of the nerve cells is the specific result of a lack of the water-soluble B. *The fat-soluble A appears to be dispensable when maintenance alone is involved, for a somewhat longer period than is the factor B.*

We base this last assertion upon the fact that pigeons can be brought into the polyneuritic state by feeding a diet free from both the essential factors A and B, and can be completely cured and maintained in a normal condition for at least 35 days on the same diet which brought on the disease, plus the water extract of a foodstuff (rolled oats) on which rats cannot grow without the addition of butter fat, but on which they do grow when the latter is added. Confirmatory evidence is also offered in our success in inducing relief from polyneuritis in birds, by treatment with water extract of wheat embryo, previously rendered lipoid-free by exhaustion with ether and carbon tetrachloride successively, and also in our success in curing birds with such substances as cabbage or potato juice, both of which are practically free from lipoids. The results with these lipoid-free products, we present

[15] McCollum and Davis, *J. Biol. Chem.*, 1915, xxi, 180. Osborne and Mendel, *J. Biol. Chem.*, 1913, xvi, 431.

[16] McCollum and Davis, *J. Biol. Chem.*, 1915, xxiii, 181, 199, Chart 5, and 221, Chart 33.

with a full appreciation of the fact that the fat-soluble A is found in considerable amount in such natural foods as wheat germ even after practically complete removal of the lipoids.

2. We also present experimental data showing that acetone, benzene, and ethyl acetate extract from wheat embryo, previously rendered fat-free by extraction with ether, the substance which relieves the symptoms of polyneuritis in pigeons.