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THE SUPPLEMENTARY DIETARY RELATIONSHIPS AMONG OUR NATURAL FOODSTUFFS*

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In order that I may illustrate the supplementary dietary relationship among certain of our naturally occurring foodstuffs, it is essential first that I offer convincing evidence that we are now in a position to devise experimental procedure which is adequate to reveal such relationships. Secondly, it must be shown that we possess sufficiently complete knowledge of the essential factors which operate in making a diet for a growing animal to enable us to interpret correctly the results of feeding experiments. This can be done perhaps best by briefly reviewing the work of the past decade which has been most significant in advancing our knowledge of the chemical complexes which are indispensable to the growing mammal.

Previous to 1909 a number of attempts had been made to nourish animals on diets composed of purified protein, carbohydrates, fats and inorganic salts, usually with a small admixture of cellulose. Such attempts were attended with failure. In every instance the cause of the failure was entirely unknown. The speculations regarding the cause dwelt principally on the dependence of the mammal on a supply of some organically combined forms of phosphorus, as phosphoprotein, lecithin and nucleic acid; its requirement of iron in some special organic form, and with the need of purin bases performed in the diet.¹ The first experimental work which proved fertile in advancing our knowledge regarding the factors which are essential in an adequate diet was that of Stepp.² He attempted to estimate the importance of lipoids in nutrition, and found that bread prepared with milk was after extraction with ether and alcohol no longer capable of maintaining life in mice. The total material extracted by the solvents, when put back into the diet, made the food efficient once more. Stepp was unable to obtain this result by the addition of any known lipoid. The latter result is significant in the light of later developments.

In 1911 Osborne and Mendel³ reported remarkable results in experiments involving growth in young rats which were fed a mixture of purified protein, carbo-

hydrate, fat, and 28 per cent. of what they termed "protein free milk," a preparation made by removing the casein of skim milk with acid and the albumin so far as possible by coagulation with heat. The "protein free" whey was evaporated to dryness. "Protein free milk" was found to induce normal growth when supplemented with purified food ingredients, and its discoverers were inclined to attribute its virtues to a nice adjustment of the inorganic constituents of the diet, although they appreciated the possible importance of unknown constituents. Two years previously Hart, Steenbock, Humphrey and I⁴ had reported most marked improvement in the capacity of cows fed on a ration restricted to the products of the wheat plant, to produce viable young, by suitable supplementing of the food mixture with certain inorganic salts, and this seemed at the time to be an adequate explanation of the good effects of feeding "protein free milk."

There were observations at this time available in the literature of pathology which were of great value in suggesting profitable lines of procedure to those who were engaged in studying the problems of normal nutrition during growth. In 1897 Eykmann⁵ had reported his observations on the production of experimental polyneuritis in fowls, analogous to human beriberi, restricting them to a diet of polished rice. He further showed that undecorticated rice could induce a cure of fowls in this condition.

Fraser and Stanton⁶ in 1907 had employed alcoholic extracts of rice polishings for the relief of experimental polyneuritis. In 1911 Funk⁷ took up the study of the problem and showed that pressed yeast, hydrolyzed with 20 per cent. sulphuric acid for twenty-four hours, still retained its property of curing polyneuritis when administered to birds. This observation disposed of the view, which had been much discussed, that some organic phosphorus compound was the curative agent. All such compounds are reduced to their simplest cleavage products by such vigorous treatment. Funk and simultaneously Suzuki⁸ and his co-workers in Japan showed that the substance which relieves polyneuritis is precipitated by phosphotungstic acid and is therefore an organic base.

It would be obvious to any one who made careful inspection of the literature up to the end of 1911 that further efforts directed toward the nutrition of animals with purified food substances must be attended with failure. As early as 1906, Hopkins⁹ had reported

4. Hart, E. B.; McCollum, E. V.; Steenbock, H., and Humphrey, G. C.: Research Bull. 17, Wisconsin Expt. Sta., 1911.

5. Eykmann, C.: Arch. f. Hyg., 1906, 58, 150; Arch. path. Anat., 1897, 148, 523.

6. Fraser, H., and Stanton, A. T.: Lancet, London, March 12, 1910, p. 733; The Etiology of Beri Beri, Study 12 from the Institute for Medical Research, Federated Malay States, 1911.

7. Funk, C.: Lancet, London, 1911, 2, 1266.

8. Suzuki, S.; Shimamura, T., and Odake, S.: Biochem. Ztschr., 1912, 43, 89.

9. Hopkins, F. G.: Jour. Physiol., 1912, 44, 425.

* Read before the Harvey Society of New York, Jan. 13, 1917.

1. The earlier experiments in this field are summarized by the author in the American Journal of Physiology, 1909, 25, 120.

2. Stepp, W.: Biochem. Ztschr., 1909, 22, 452; Ztschr. f. Biol., 1912, 57, 135; ibid., 1912-1913, 59, 366.

3. Osborne, T. B., and Mendel, L. B.: Publications of the Carnegie Institution of Washington, Bull. 156, Pt. 2, 1911.

his observation that the addition of small amounts of milk to food mixtures consisting otherwise of purified protein, fats, carbohydrate and salt exerted a favorable influence on nutrition which was out of all proportion to the calorific value of the added milk, and he expressed the belief that there existed certain "accessory food substances" indispensable from the diet. These observations convinced me of the importance of further examination of the lipid moiety of the diet. In 1912 Miss Davis and I observed the remarkable stimulating action on growth of purified butter fat and of the ether extract of egg yolk, when these were added to a mixture of supposedly purified food substances. Lard and olive oil possessed no such properties.¹⁰

The situation was still not clear. In June, 1912, Osborne and Mendel¹¹ described experiments in which normal growth curves were secured with rats over a period of sixty days, on a ration consisting of pure protein, starch and "protein free milk," all the constituents being free from substances soluble in ether, and practically free from lipids of any character.

One phase of the difficulty was cleared up by our observation that when lipids of suitable character were present in the food mixture, we secured growth when 20 per cent. of the diet consisted of nearly nitrogen free lactose (Merck or Kahlbaum), but were unable to do so when the latter was replaced by starch.¹² Obviously an impurity in the lactose furnished a second unknown dietary factor without which growth could not proceed. The inclusion of lactose with the omission of the fats of milk or egg yolk was, however, attended with failure to secure sustained growth. This was difficult to understand in the light of Osborne and Mendel's temporary success in inducing growth by the employment of ether extracted "protein free milk." The explanation is now available. Simmonds, Steenbock and I¹³ have found that the fat-soluble dietary essential is appreciably soluble in water, and that repeated washing of butter fat with distilled water removes from it the substance which is responsible for its peculiar effect in the diet. The water solution of the nonlipoid constituents of milk, therefore, contain this substance, indeed, in considerable amount. Recent experiments lead us to believe that approximately half of the fat-soluble dietary essential is present in the fat and half in the nonfat portion of the milk. It is, therefore, approximately thirty times as soluble in butter fat as in water. "Protein free milk" contains, therefore, both the water and alcohol-soluble dietary essential which is found in many natural foodstuffs and which is the active agent in the cure of polyneuritis, and a small amount of the fat-soluble essential as well.

Observations such as I have described led Miss Davis and me¹² in 1915 to propose the working

hypothesis that an adequate diet during growth must furnish, in addition to the well recognized constituents of the diet—protein, carbohydrate, fats and inorganic constituents of adequate character and amounts—two as yet unidentified dietary factors. One is soluble in water and alcohol and apparently never associated with the lipoids, when these are isolated from natural foodstuffs, the other soluble in fats. The latter is extracted from milk, egg yolk, kidney and probably other animal tissues by ether, but is not removed by this solvent from either the seeds or the leaves of plants.¹⁴ The former is universally present in foodstuffs of vegetable and animal origin, but is absent or nearly so from crystalline sugar, starch and fats, and is present in but small amount in polished rice and probably in those foods derived from the endosperm of seeds by milling processes. Where the reaction of the medium is alkaline, strong heating of foods probably leads to its destruction.

Stepp failed to interpret his observations correctly because hot alcohol takes out both of these dietary factors, and when he put back the extract with the extracted residue, both were restored.

Osborne and Mendel could not correctly interpret the cause of their success with "protein free milk" combined with purified foodstuffs because they were adding both these unknown substances, although the amount of the fat-soluble one was inadequate to serve throughout the life of the animal.

I am frequently confronted with the questions: What of the several "vitamins" which have been so eagerly seized on of late to explain such pathologic manifestations as beriberi, scurvy, pellagra and rickets?

Do not these several well recognized syndromes, the etiology of each of which is generally believed to be more or less directly referable to inadequate diet, connote the existence of as many "vitamins," each specific in the protection it affords against a type of abnormal metabolism? I am almost convinced that there are but two individual substances, the solubilities and distribution of which I have just described, which constitute the only essential dietary ingredients with whose chemical natures we are not fairly familiar. The possibility remains, of course, that one or both of what I have termed the fat-soluble A and water-soluble B each represent more than a single substance. I shall speak further on this point later.

Employing this working hypothesis, we have examined the nature of the dietary deficiencies of several of the more important natural foodstuffs. The procedure in each case was as follows: The individual foodstuff, maize,¹⁵ for example, we fed (a) as the sole source of nutriment; this leads to complete failure of young animals to grow, and death usually supervenes within two months; to another group (b) we fed maize plus butter fat to furnish the unidentified dietary factor A; (c) maize plus a liberal amount of a complete protein, casein; (d) maize plus a salt mix-

Chart 1.—Results of feeding animals on maize with purified food substances. In this and the following charts, Y marks the birth of young.

10. McCollum, E. V., and Davis, M.: *Jour. Biol. Chem.*, 1913, **15**, 167; *ibid.*, 1915, **23**, 231.

11. Osborne, T. B., and Mendel, L. B.: *Jour. Biol. Chem.*, 1912, **12**, 81.

12. McCollum, E. V., and Davis, M.: *Jour. Biol. Chem.*, 1915, **23**, 231.

13. McCollum, E. V.; Simmonds, N., and Steenbock, H.: *Proc. Am. Soc. Biol. Chemists*, December, 1916; *Jour. Biol. Chem.*, March, 1916.

14. McCollum, E. V.; Simmonds, N., and Pitz, W.: *Am. Jour. Physiol.*, 1916, **41**, 333, 361.

15. McCollum, E. V.; Simmonds, N., and Pitz, W.: *Jour. Biol. Chem.*, 1916, **28**, 153.

ture so constituted as to supplement the inorganic content of maize and give the animal an inorganic supply similar to that which is supplied by milk, a food on which good nutrition with growth is attained. As will appear later, maize contains a liberal amount of the water-soluble B. In all these experiments the behavior of the animals was substantially the same. There was little growth in any case. Of the three additions described, the proper adjustment of the inorganic content is the most beneficial (Rat 815, Chart 1).

Obviously more than a single dietary factor is at fault in determining the failure of young rats to grow on maize. We conducted a series of experiments in which maize was fed with two purified food additions: (e) maize, salts and casein; (f) maize, salts and butter fat; (g) maize, butter fat and casein. The results of feeding such diets are shown by the curves in Chart 1. Rat 569 made somewhat less than half the normal rate of growth over a period of five months when both the protein and inorganic content of maize were supplemented. The addition of butter fat at this time caused extremely rapid growth, and during the following three months two litters of young were born, one, the second, being successfully reared. Rat 619, which received maize, salts and butter fat, grew slower than the normal rate and showed decline after the age of 6 months. She never produced young. Rat 618, which was given maize, casein and butter fat, did not grow at all during a period of six weeks. Four per cent. of a suitable salt mixture was then added, with the result that growth was promptly resumed and the animal reached the full adult size at the normal rate and produced three litters of young, all of which were successfully weaned. These records are each representative of a group of rats.

TABLE 1.—COMPOSITION OF SALT MIXTURES

Salt mixture used with Rats 223, 223B and 380 (Fig. 2).	
Sodium chlorid (NaCl)	0.668
Dipotassium phosphate (K_2HPO_4)	0.337
Monocalcium phosphate ($CaH_2(PO_4)_2 \cdot H_2O$)	0.284
Magnesium sulphate (anhydrous) ($MgSO_4$)	0.018
Magnesium citrate ($Mg_3(C_6H_5O_7)_2 \cdot 14H_2O$)	0.068
Sodium citrate (anhydrous)	0.037
Ferrous lactate	0.119
Potassium citrate	0.799
Calcium lactate	2.533
Salt mixture 185 (Figs. 1, 4, 6, 8, 10)	
Sodium chlorid (NaCl)	0.173
Magnesium sulphate (anhydrous) ($MgSO_4$)	0.266
Monosodium phosphate ($NaH_2PO_4 \cdot H_2O$)	0.347
Dipotassium phosphate (K_2HPO_4)	0.954
Monocalcium phosphate ($CaH_2(PO_4)_2 \cdot H_2O$)	0.540
Ferrous citrate	0.118
Calcium lactate	1.300
Salt mixture 500 (Figs. 3, 6, 8)	
Sodium chlorid (NaCl)	0.5148
Calcium chlorid ($CaCl_2$)	0.2569
Dipotassium phosphate (K_2HPO_4)	0.3113
Potassium citrate	0.5562
Calcium lactate	2.878
Salt mixture 318 (Figs. 1, 2, 6)	
Sodium chlorid (NaCl)	1.400
Dipotassium phosphate (K_2HPO_4)	2.531
Potassium citrate	0.710
Calcium sulphate ($CaSO_4 \cdot 2H_2O$)	0.578
Calcium lactate	7.058

Chart 2 illustrates the behavior of rats with respect to growth and reproduction when fed wheat with two

purified food additions,¹⁶ casein and salts, Rat 223; casein and butter fat, Rat 380, and butter fat and salts, Rat 319. Rats in Lot 223 in some instances grew rapidly to full adult size, but all were short lived. At the age of 6 months they were all emaciated and showed edema of the eyelids with hemorrhage of the conjunctiva. In Rat 380, which received wheat, casein and butter fat, growth was very slow. After being stunted during five months, on the addition of a suitable salt mixture the animal experienced a wonderful acceleration of growth and complete recovery from a condition of partial baldness, accompanied by a roughness of the skin and tail. Animals in this group have frequently suffered from a purulent bronchitis.

The stunted growth, lack of fertility and early decline which follows the feeding of wheat with salt and butter fat additions is illustrated by Rat 319. When, however, wheat is supplemented with three purified additions, casein, salts and butter fat, it becomes capable of supporting growth, reproduction and rearing of the young in a manner which closely approaches the optimum, as is shown by the curve of Rat 223 B.

Wheat and maize are therefore closely similar in their dietary properties. Each requires an improvement in its protein moiety, a suitable supplementing of its inorganic content and an added supply of the

dietary factor which I have designated the fat soluble A. Each contains an abundance of the second unidentified dietary essential, the water soluble B, since without any addition of this substance, excellent nutrition can be secured.

Let us consider for a moment the dietary properties of the oat kernel¹⁷ as revealed by feeding it supplemented with single or multiple additions of purified foodstuffs. It becomes apparent from an inspection of Chart 3 that the same general relations hold as we have seen in the case of the maize and wheat kernel. The curves are each representative of the performance of a group of not less than four rats. Here, however, the addition of casein, salts and butter fat does not lead to normal nutrition. We have not yet been able to supplement oats with purified food ingredients and attain optimum results, when the oat kernel constituted from 70 to 80 per cent. of the food mixture. Gelatin combined with oat proteins forms a much better protein mixture than do casein and oat proteins. This will be made apparent by an inspection of Chart 6, Rat 647. (Compare Charts 6 and 3.) We have not yet determined the cause, but it is evident that a high intake of oats over a long period causes injury to the rat. This is true also for the cow, and I believe also for swine.

The results of testing combinations of two or more kinds of seeds as a restricted diet has proved extremely interesting. The curves of four representative rats

16. Hart, E. B., and McCollum, E. V.: Jour. Biol. Chem., 1914, 19, 373. McCollum and Davis: Ibid., 1915, 21, 615. Hart, E. B., Miller, W. S., and McCollum, E. V.: Ibid., 1916, 25, 239.

17. McCollum, E. V.; Simmonds, N., and Pitz, W.: Unpublished data.

receiving diets made up on this plan are shown in Chart 4.¹⁷ It proved a great surprise that we were not able to make combinations of the seeds of plants in any way which forms a ration capable of inducing normal growth in the rat, and an extensive experience has all tended to confirm our belief that this is true for swine and probably for other mammals as well. It is possible, however, with rations restricted to seeds, to secure nutrition of at least a fairly satisfactory character with pigeons¹⁴ and possibly also with chickens. Chart 4 shows clearly the dietary factor which forms the most important cause in inhibiting the growth of the rat on these diets. It is the character and amount

and is of an entirely different character from that of the seed. In Table 2 are given the average analyses of alfalfa hay, cabbage, wheat, oat and corn kernel, potato and milk.

It is fortunate, indeed, that the inorganic content of the seed and the leaf supplement each other in a manner which meets the demands of mammalian physiology. The second way in which the leaf supplements the grain is in respect to the as yet unidentified dietary factor, fat-soluble A. This substance is present in the alfalfa leaf to the extent of at least five times the amount in the maize kernel, so that any diet containing 30 per cent. or more of this leaf is not enhanced by the inclusion of butter fat. Cabbage and clover leaves also are rich in this dietary essential, but our experience with these is less extensive. There is to be expected, of course, a supplementary relationship among the proteins of a mixture of two or more foodstuffs. It could hardly happen that the proteins from several sources would be particularly low in their content of the same amino acids. That the limiting amino acid is not the same in several of the more important seeds is made evident by the curves

shown in Chart 6. Rats 652 and 493 received a similar food mixture, except that the former had 10 per cent. of gelatin supplementing wheat proteins in place of an equivalent amount of dextrin. The gelatin-containing

Chart 3.—Results of feeding animals on oats with purified food substances.

of the inorganic portion of the food mixture. When this is properly adjusted by means of salt additions, growth can proceed on certain mixtures. After prolonged suspension on Ration 713, the addition of salts led to growth. Equally pronounced failures with rations of this type containing beans or peas are abundant in our records.

In marked contrast to our failure to secure satisfactory nutrition with any of the many combinations of from two to five kinds of seeds stand the nearly normal growth of rats which are limited from an early age to a monotonous mixture of a seed and a leaf. We have tested most thoroughly combinations of the alfalfa leaf with several seeds, since it can be secured as a meal and as a flour, the former being much employed in animal production. The products which we have employed were very fine meal consisting principally of the leaves, but containing visible pieces of stem, and also a flour which was practically entirely derived from leaves. Both products came from immature plants dried in the sun.

Chart 5 illustrates the fact that rats can take from the age of 35 days a mixture of 60 per cent. of any one of at least three seeds, oats, wheat and maize,¹⁷ with 40 per cent. of alfalfa leaves, and grow to a size closely approximating the normal, and produce young. The same proportions between polished rice and alfalfa,¹⁴ peas and alfalfa,¹⁶ and cottonseed flour and alfalfa¹⁷ yield less satisfactory results, but with these growth is far superior to what we have ever observed with the seeds alone in any combinations or proportions, when fed to rats whose water supply was distilled, and therefore salt free.

On Ration 685, oats and alfalfa,¹⁷ fourteen out of the seventeen young were reared. The one litter of five produced by Rat 687 all died soon after birth. Four of the seven young in the litter from Rat 686 were reared. No young have been reared on the polished rice and alfalfa mixture (Rat 478).

Two factors contribute toward making these combinations of seed and leaf superior to seeds alone. First, the inorganic content of the leaf is much greater

TABLE 2.—AVERAGE ANALYSES OF VARIOUS FOODS IN ONE HUNDRED PARTS OF DRY SUBSTANCES*

	Total Ash	K	Na	Cu	Mg	Cl	P	S
Alfalfa..	7.380	1.041	0.007	2.146	0.223	0.208	0.338	0.172
Cabbage	10.850	2.510	0.805	1.063	0.234	0.853	0.853	0.372
Wheat....	2.080	0.504	0.020	0.041	0.141	0.058	0.408	0.009
Oat.....	1.890	0.361	0.058	0.007	0.080	0.098	0.385	0.005
Corn.....	1.450	0.358	0.012	0.023	0.130	0.013	0.289	0.005
Potato...	3.70	1.860	0.083	0.072	0.113	0.131	0.270	0.009
Milk.....	5.07	1.160	0.344	0.009	0.088	0.701	0.050	0.057

* From Forbes, Bull. 207, Ohio Expt. Station.

Chart 4.—Failure of rats to grow when restricted to a diet of seeds. The limiting factors are the inorganic content and the fat soluble A.

diet promoted growth at a rate greater than the normal expectation, as compared with a rate about half normal in the gelatin-free control.¹⁸

The second pair of curves, Rats 756 and 785, received rations alike in all respects, except that in the former the protein content (19.9 per cent.) was derived solely from a mixture of peas and gelatin¹⁷ and the latter from a mixture of navy beans and gelatin.¹⁷ In neither do the rations promote appreciable growth, although the entire group receiving beans grew noticeably better than did those getting peas. Gelatin does not supplement efficiently the proteins of either the pea or bean. In the second period in each

18. McCollum, E. V.; Simmonds, N., and Pitz, W.: Jour. Biol. Chem., 1917, 28, 483.

case casein was substituted for the gelatin with the result that growth began at once at a fairly good rate. The proteins of the pea or the bean, when taken as the sole source of nitrogen, are of very low biologic value.¹⁷

An inspection of the curves of Rats 647 and 646 shows that gelatin has a very favorable supplementary relation to the oat proteins.¹⁸ Gelatin when combined with the proteins of the maize kernel does not enhance the value of the latter.¹⁸

In 1914 we repeated the efforts of Slonaker¹⁹ to find the effects of a strictly vegetarian diet of wide variety, on the growth of the rat. We offered nineteen differ-

bean proteins as contrasted with peas. Pea proteins are wasted when combined with those of the oat, while the proteins of the navy bean and oat mutually make good each other's deficiencies.¹⁷ Rats 782 and 781. It follows, therefore, that the proteins of the pea and the bean are very dissimilar in respect to their yields of certain amino acids, and that the same amino acid is not the limiting factor in each.

In Chart 9 are presented curves obtained with a series of diets designed to show the relative richness of the fat-soluble A in several natural foodstuffs. The diet, aside from its content of natural food, consisted of such a mixture of purified food materials as would support growth, when both the unidentified dietary factors, the fat-soluble A and water-soluble B, are added. The rôle of the natural food in these rations was to supply these two factors.¹⁷ These curves make it clear that about 30 per cent. of alfalfa leaf is the minimum amount which will supply the fat soluble A in amount sufficient to induce complete growth and induce the production of a few young. The young were not reared on this ration.

In Charts 1, 2 and 3, curves were shown which illustrate that even 70 per cent. of corn, wheat or oat kernel do not furnish enough of the dietary A to support complete growth and repeated reproduction and well-being over the full span of life.

It is of particular interest, therefore, to compare the effects of flaxseed and millet seed as sources of the fat soluble A. Both of these are richer in this substance than are the cereal grains, and millet seed proves to be unique among the seeds we have examined as a good source of this dietary factor. When 25 per cent. of millet seed was combined with purified foodstuffs, growth was completed and one rat has produced two litters of young. After eight months on this diet

Chart 5.—Good growth promoting ration which a combination of one leaf and one seed may produce.

ent foods of vegetable origin, giving the animals five or six at a time and changing their bill of fare at intervals of a few days. The list of foods included grains, leaves of plants, nuts, corn and wheat germ, peas and flaxseed meal. None ever grew beyond half the adult size.¹⁴ We duplicated in all essentials the results which Slonaker had observed. Having gained some insight into the peculiar dietary properties of certain natural foods, I predicted two years ago that a mixture of maize 50, alfalfa leaves 30 and heated peas 20 would adequately nourish a young rat during growth. A trial proved that this strictly vegetarian food mixture induced satisfactory nutrition during growth, and supported repeated reproduction and rearing of a fairly large percentage of young.¹⁴ Chart 7 shows the curves from left to right of mother, daughter and granddaughter. A litter of great grandchildren were successfully weaned and appeared entirely normal when we discontinued the experiment. I should like to emphasize the importance of a proper adjustment of the well recognized dietary factors by pointing out that no young would be reared if this diet were changed to include less than 20 or more than 45 per cent. of alfalfa, the maize replacing or being replaced by alfalfa in the adjustments.

In Chart 8 are shown representative curves showing the failure of the proteins of either peas or navy beans to supplement very efficiently the proteins of the maize kernel.¹⁷ Rats 779 and 780. In either of these rations the peas or beans could be replaced by dextrin, and a curve of nearly the same shape would be secured. Both pea and bean proteins improve the proteins of the wheat kernel,¹⁷ Rats 783 and 784, for these curves are much better than would be secured if the legume seed were replaced by carbohydrate (Chart 1, Rat 619; Chart 2, Rat 319). The most remarkable result of studying these combinations is seen in the very different results of supplementing oat proteins with

Chart 6.—Value of gelatin in supplementing the deficiencies of the wheat and oat kernel, but not those of maize, beans or peas.

she appears to be in perfect nutrition. The slow but long continued growth on these rations, whose content of both unidentified dietary factors was solely derived from such small amounts of hemp seed, flaxseed and millet seed, shows that these stand intermediate between the cereal grains on the one hand and the plant leaf on the other as sources of the fat-soluble A.¹⁷

We have carried out a series of experiments designed to show the minimum amount of several seeds which are necessary to supply enough of the water-soluble dietary essential for the completion of growth and the production of young. Chart 10 illustrates the results of this inquiry. The diet in all cases consisted of a purified food mixture, to which was

19. Slonaker, J. R.: Leland Stanford Junior Univ. Pub. Univ., Series 1912.

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extracts which contain the dietary factor B is equally certain. Much less definite statements can be made regarding the other diseases, rickets and pellagra, which Funk has attributed to lack of specific substances in the diet. The evidence, as I understand it, seems to point strongly toward diet as at least an important contributing factor. Assuming that they as well as beriberi are of nutritional origin, I venture to state that they are the result of unsatisfactory adjustments among the well recognized constituents of the diet rather than to the lack of specific chemical substances. I have shown how diets may be so designed as to induce faulty nutrition as the result of the shortage of each of the two unidentified dietary factors A and B; of the inadequacy of the protein content, because of an unsatisfactory yield of amino acids and as the result of an unsatisfactory composition or inadequate amount of the inorganic content of the food mixture. I may add to these a fifth, namely, malnutrition as the result of the presence of toxic substances in the natural foodstuffs, particularly cottonseed and wheat products, and perhaps also a sixth cause due to mechanical injury to the digestive tract caused by foods (for example, oats) producing feces of unfavorable character, and by constant distention of the alimentary tract as the result of excessive fermentation of foods, such as beans and peas, which contain a large amount of hemicelluloses.

I have attempted to show, so far as experimental data are available, the specific dietary properties of our natural foods, and how these may be so combined as to make good each other's deficiencies and shortcomings. In presenting these data it has also been emphasized how great is the need of more knowledge in this field. It is indeed remarkable how difficult it is to secure combinations of natural foods which will serve as monotonous diets over a long period and promote in an entirely satisfactory way the well-being of an animal. The consumption of a varied diet makes in some degree for safety, but will by no means insure safety. It remains to be seen whether or not the animals which we have brought to a condition of nutritive disaster by a series of diets, each of which was faulty in respect to but a single dietary factor, as inorganic salts or protein, or the unknown A or B, will reveal to the histologist and pathologist lesions analogous to those seen in the states of human malnutrition presumably of dietary origin. The inadequate diets of man are probably never highly satisfactory with respect to all factors but one. Rather they are in some degree unsatisfactory with respect to two or three dietary factors simultaneously, and the probability that they will be so increases greatly as the food supply tends to be limited to the seeds of plants, and such manufactured products as are derived from the endosperm of seeds. The time will doubtless come within a few years when specific advice can safely be given as to the best way to plan a variety of human dietaries; but a consciousness of the paucity of our knowledge concerning the peculiar supplementary relationships among our natural foodstuffs forbids more than a general statement as to the safest policy now.

A diet in which the seeds of plants form the principal part will be made more safe by the judicious use of milk and of the leaves and probably also of the tubers of plants. These have peculiar dietary properties which the best chemical talent of today fails to recognize, but which are readily demonstrable by biologic tests.

APPLICATION OF THE COMPLEMENT-FIXATION TEST TO TUBERCULOSIS

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During recent years much experimentation has been carried out in determining the value of complement-fixation reactions for the diagnosis of tuberculosis.

Results obtained by different workers show considerable variation. When, however, it is remembered that the value of the test is entirely dependent on the specific action of the antigen and that the antigens used by the many investigators have been prepared by widely divergent methods, it is to be expected that the results should be somewhat dissimilar. At best, we are but feeling our way toward a common understanding of the relation which our laboratory findings bear to the disease itself, and the test will eventually be established as of practical value only by a thorough comparison of all careful investigations rather than by the consideration of the work of any one person.

Several experimenters have obtained results of much promise, but on account of cross fixations with syphilitic serums or because of positive reactions in a certain percentage of nontuberculous cases, the test has not seemed sufficiently reliable to warrant its ready adoption as a routine test in diagnostic laboratories.

Miller and Zinsser¹ of Columbia University, New York, claim an accuracy of nearly 100 per cent. for complement fixation in the detection of active tuberculosis. They used an antigen made up of ground tubercle bacilli in physiologic sodium chlorid solution.

Preparation of this antigen is thus described:

"Twenty mg. of the moist tubercle bacillus mass are weighed out, placed in a conical 15 c.c. centrifuge tube, and to it are added 90 mg. of table salt. With a glass rod, filed to roughness at the end, this paste is ground by hand for about one hour. Distilled water is then added to isotonicity, that is, 10 c.c. to the quantities described above. This is the antigen. Just before using, it is shaken up and the heavier particles are allowed to settle in the course of a few minutes. Except for the removal of these larger elements, the suspension is used as a whole without centrifugation and without filtration."

It seemed to us that it would be of much interest and value to run a series of tests with the Miller antigen since the Miller figures, if confirmed by other investigators, place the test on as accurate a basis as can be claimed for the Wassermann reaction.

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1. Miller and Zinsser: Proc. Soc. Exper. Biol. and Med., 1916, 13, 134-138.