AMINO-ACIDS IN NUTRITION AND GROWTH.[1]

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The current trend of the investigation of the chemistry of nutrition is emphasizing the significance of the amino-acids as the fundamental factors in all problems in which hitherto the rôle of proteins has been involved. The remarkable success which has attended the efforts to supplant completely the proteins in the food intake by their ultimate products of hydrolysis—the so-called amino-acid "Bausteine"—has led to promising researches in which these food fragments have been followed beyond the alimentary barrier into the blood stream, to the tissues, and almost to their final destruction in the body. The question of protein synthesis has now become a problem of the biochemical deportment of amino-acids.

By Abderhalden's well-known experiments in this field of study it has been demonstrated that dogs (and even man) can be maintained in nitrogenous equilibrium, or, in some instances, can even gain weight and retain nitrogen when completely digested protein supplies the nitrogenous component of the diet.[2] Furthermore

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] The earlier literature is referred to in Abderhalden's *Synthese der Zellbausteine in Pflanze und Tier*, Berlin, 1912; Abderhalden: Fütterungsversuche mit vollständig abgebauten Nahrungsstoffen, *Zeitschr. f. physiol. Chem.*, lxxvii, pp. 22–58, 1912; cf. also the experience of Henriques and Anderson: Über parenterale Ernährung durch intravenöse Injektion, *Zeitschr. f. physiol. Chem.*, lxxxviii, pp. 357–369, 1913, in which similar results were obtained by intravenous infusion of nutrients including proteins that were digested by trypsin and erepsin.

nutritive equilibrium has been obtained during short periods by furnishing an artificial mixture of isolated amino-acids in place of the dietary protein.

There exists, today, a body of evidence indicating that not all of the known amino-acids need to be furnished in the diet in order to secure maintenance or even growth. Glycocoll, for example, may be missing entirely, and it is probable that the animal organism can synthesize it anew. Abderhalden[3] has suggested that proline is not an indispensable amino-acid in maintenance, and that the organism may be able to form it from glutaminic acid. He also intimates that arginine may be replaced by ornithine, which readily unites with urea to form arginine. With respect to the possibility of a synthesis of such structural units as alanine, valine, leucine, etc., by the animal organism no positive statement can be made today. On the other hand it is clear that when certain amino-acid groups are lacking, nutritive equilibrium is impossible. The cyclic derivatives, tyrosine and tryptophane, appear to be included herein. From this viewpoint the long known incapacity of proteins like gelatin and zein, which are deficient in one or more supposedly essential amino-acids, to satisfy the nutritive needs of the body in respect to protein, are easily interpreted as failures due to the lack of the missing "Bausteine" which animals are incapable of furnishing by direct synthesis. We have hitherto referred to the hypothesis of W. A. Osborne that "cyclopoiesis," in the sense of synthesis of cyclic groups in amino-acids, is a property of the plant cell, the animal organism being "acyclopoietic" and to this degree dependent on plant life for certain types of essential nutrient complexes.

In obtaining criteria as to precisely what amino-acids are essential in the sense that they must be supplied to the organism because they cannot be manufactured *de novo* by it, much of the accumulated experimental evidence falls short of absolute proof. Prolonged growth is an admirable index of protein synthesis. Gains or losses in body-weight after addition or subtraction of amino-acids from the intake mean little unless they are extended over long periods. One needs only recall that material gain in weight can be made for a time on a nitrogen-free diet rich in carbohydrates and

[3] Abderhalden: *Zeitschr. f. physiol. Chem.*, lxxvii, p. 27, 1912.

fats, and that animals can gain weight while constantly losing nitrogen. The reserve capacity of the animal body in respect to essential compounds must likewise not be overlooked. The possible importance of this factor derives emphasis from the records of the long periods of starvation—over one hundred and seventeen days in the case of the dog—which have been survived successfully. In the face of such facts one may well hesitate to offer any final pronouncements regarding the indispensability, or the synthesis, of some particular amino-acid, when the conclusion is based upon experiments of a few days' duration at most.

The preceding considerations bear upon what constitutes evidence of whether an amino-acid is essential or not, in the sense that it cannot be manufactured in the animal organism and must accordingly be supplied in the intake if nutrition adequate for the existing condition of the individual is to be maintained. There are further features of the nutritive needs of the body which have been inadequately appreciated and not clearly formulated in the past. Emphasis has frequently been laid upon the differences in the requirements for so-called maintenance and those for growth; for the most part the distinctions brought out, at least in the case of the protein requirement, have been quantitative rather than qualitative in character. We are told that a certain quota of the protein intake is necessary to replace the "wear and tear" of the organism. This is regarded as a function of the intensity of the life processes, being proportional to the amount of protoplasmic material present, provided nutrients other than protein are furnished to cover the requirement for energy. Otherwise, if protein be ingested it will itself be used to supply the energy requirement. These ideas, formulated by Rubner[4] in regard to the protein requirement of the cells, have been summarized by Lusk as follows:

> The "wear and tear" quota must be covered by the ingestion of the "repair" quota. Ingestion of protein beyond the amount needed for the repair of the normal waste may first be used for growth. The portion used for growth is called "growth" quota and is dependent on the protein condition of the cells. The conditions which determine the "wear and tear" metabolism and those which determine growth are entirely dissimilar,

[4] Rubner: *Arch. f. Hygiene*, lxvi, p. 45, 1908.

although without metabolism growth is impossible. If protein be administered beyond the requirement of the cells for repair and for growth, then the excess constitutes a "dynamic" quota which after deamination serves to furnish energy to the cells in the same fashion as do fat and sugar.[5]

In previous publications[6] we have presented clear evidence for the first time, we believe, that quite aside from the energy aspect and the quantitative features of the diet, certain proteins, notably the gliadin of wheat, may supply the nitrogenous needs of an animal in maintenance, yet be entirely inadequate for the purposes of growth. Still other proteins, as has long been known of gelatin and zein, fail to permit even the maintenance of animals in nutritive equilibrium. The remarkable fact that an animal can be maintained satisfactorily over very long periods, not merely a few days or weeks, on a diet which promptly becomes a promoter of growth by the mere substitution of another protein for gliadin, raises the question whether, after all, protein in the broader sense is necessarily destroyed by the so-called "wear and tear" of the body. The energy supply of the body can doubtless be obtained from any type of digestible food-stuff. Let us assume, for the purpose of argument, that a few definite amino-acids are indispensable in the functioning of certain cells or glands of the organism—that tryptophane, for example, is essential to the activities of some organ of internal secretion or in the elaboration of a necessary hormone, as already suggested by Willcock and Hopkins.[7] Tryptophane cannot be synthesized by the animal cells. It must therefore be furnished preformed to the individual, or obtained by decomposition of its tissue proteins, to permit the life activities to continue. Ordinarily this is accomplished by ingestion of tryptophane-yielding proteins. In accord with such a hypothesis it is

[5] Lusk: *The Elements of the Science of Nutrition*, 2d edition, 1909, p. 187.

[6] Osborne and Mendel: Beobachtungen über Wachstum bei Fütterungsversuchen mit isolierten Nahrungssubstanzen, *Zeitschr. f. physiol. Chem.*, lxxx, pp. 307–370, 1912; The Rôle of Gliadin in Nutrition, this *Journal*, xii, pp. 473–510, 1912; Maintenance Experiments with Isolated Proteins, *ibid.*, xiii, pp. 233–276, 1912; Wheeler, Ruth: Feeding Experiments with Mice, *Journ. of Exp. Zoölogy*, xv, pp. 209–223, 1913; Mendel: The Rôle of Proteins in Growth, *Trans. XV Internat. Cong. on Hygiene and Demography*, 1912.

[7] Willcock and Hopkins: Observations on the Effect of Adding Tryptophane to a Dietary in which Zein is the Sole Nitrogenous Constituent, *Journ. of Physiol.*, xxxv, pp. 88–103, 1906.

quite conceivable that what has hitherto been called the "wear and tear" (Abnutzungsquote) is in reality the demand for an amino-acid necessary for the construction of some non-protein nitrogenous substance essential for normal metabolism, rather than a requirement for the replacement of all the "Bausteine" which go to make up cellular proteins. On the basis of present evidence it is quite as logical to assume that the maintenance protein requirement is in reality a requirement for definite (as yet specifically undetermined) amino-acids that serve special physiological functions, as it is to insist that protein as such is demanded to repair a hypothetical destruction of the entire protein molecule. The latter may be regarded as ordinarily undergoing degradation only because by this method some essential amino-acid is liberated. Evidently zein and gelatin do not contain this; hence they cannot replace this non-protein nitrogenous wear and tear and consequently do not suffice for maintenance. Gliadin does contain the essential complex. The conspicuous qualitative distinction between the proteins referred to is the presence of the tryptophane group in gliadin and its absence from zein and gelatin. As we shall show, an animal can be maintained at constant weight for relatively long periods on a food containing gliadin as its sole protein factor, and at once be made to grow by adding lysine to such foods. Since synthesis of new protein is essential for the construction of new tissues, growth will be limited by any factor which prevents the synthesis of protein—in the above case, lysine. If such an interpretation of this failure to grow is correct, we should expect that these defective foods would fail to maintain the body-weight if protein is destroyed in the metabolism of maintenance unless the essential amino-acids can be synthesized as such. Inasmuch as animals on the above diets do not lose weight it is not improbable that very little, if any, protein is destroyed in such maintenance, and that the defective proteins furnished in these foods serve some other purpose than the reconstruction of protein (cf. Chart I, Rat 1113).

In respect to the nitrogenous requirements of the organism, growth sets a standard decidedly higher than that of maintenance; for certain amino-acids which cannot be synthesized by the growing organism must be furnished, not only for the maintenance functions but also for the construction of new tissue.

In respect to the precise character of the evidence which would be convincing in relation to protein synthesis from the amino-acids of the food, Abderhalden and Oppler wrote in 1907:

> The problem of protein synthesis in the animal body from the simple "Bausteine" could be solved beyond criticism, if it were possible to cause young animals to increase in weight rapidly, and thereby produce an increase of their tissues, by feeding completely digested protein. Manifold obstacles interfere with such an experiment, and only an extensive experience will make it possible to furnish a convincing demonstration.[8]

Although Abderhalden and his collaborators have repeatedly referred to evidence of a new construction of tissues in some of their experiments in nutrition with amino-acids as the source of nitrogen, the actual gains reported are of a far different magnitude than those which are observed in normal growth such as, for example, we have repeatedly demonstrated in our studies on rats fed upon mixtures containing isolated proteins, and in those which form the subject of the present paper. This need not be interpreted as contradicting in any way the contention that growth, *i.e.*, a new synthesis of tissue protein, is possible from suitable intake of amino-acid mixtures; it merely means that more convincing data are still desirable. The best previous record which we recall was obtained by Abderhalden and Hirsch[9] who secured an increase equal in one case to 26 per cent of the original weight of the experimental animal, by feeding "erepton"—a digestion product prepared from meat. Abderhalden also states that in some of his experiments young dogs on diets of predigested foods have made considerable gains in weight—in one case 1000 grams, in another 1200 grams.[10] From this he argues that new formation of tissue has proceeded in considerable degree, and that the animal organism is able to construct all its cell components from simple "Bausteine." No temporary or transient gain of weight, and none which follows the depletion of the body by previous unsuitable nutritive conditions,

[8] Abderhalden and Oppler: *Zeitschr. f. physiol. Chem.*, li, p. 232, 1907.

[9] Abderhalden and Hirsch: Fütterungsversuche mit Gelatine, Ammonsalzen vollständig abgebautem Fleisch und einem aus allen bekannten Aminosäuren bestehenden Gemisch ausgeführt an jungen Hunden, *Zeitschr. f. physiol. Chem.*, lxxxi, pp. 323–328, 1912.

[10] Abderhalden: Fütterungsversuche mit vollständig abgebauten Nahrungsstoffen, *Zeitschr. f. physiol. Chem.*, lxxvii, pp. 22–58, 1912.

can be taken as evidence of true growth, for repair may be accomplished without necessarily implying actual synthetic processes in the sense intended. Real growth, consistently continued, manifests itself in characteristic increments of weight and size as exhibited in typical curves of growth.

It has been cleverly stated that "the tissue cells never know the food we eat." If the amino-acids are the true nitrogenous nutritive agents, and the ingested proteins appear to be equivalent in their nutritive efficacy for maintenance or growth to the amino-acids which they yield, the proteins themselves may be compared strictly from this viewpoint. In the light of present-day information the failures to replace protein by gelatin in this way have been due to inadequate knowledge in respect to the nature and proportions of the amino-acids which are linked together in the gelatin complex. Prior to the discovery of tryptophane the neglect to supply this derivative inevitably resulted disastrously; and even today, despite the recognition of the further lack of cystine and tyrosine, the composition of gelatin is not adequately known. The demonstration of the possibility of supplementing gelatin with amino-acids so as to render it complete in the sense of promoting growth remains to be accomplished. In so far as their chemical make-up is adequately known, the complete lack of, or partial deficiency of, individual proteins in any amino-acid can be supplemented by artificial additions to the imperfect protein, precisely as has been done in the case of inadequate mixtures of amino-acids. The addition of such amino-acids as are missing, or deficient in amount, ought, on this hypothesis, to make it possible to render adequate all proteins which are digestible in the alimentary tract. The history of earlier experiences in this field—of the attempts to supplement the then known deficiency of gelatin in particular by admixture of tyrosine, etc., in the hope of rendering it adequate for nutritive purposes, need not be detailed here.[11]

[11] See Escher: *Vierteljahresschrift der Naturforscher Gesellschaft in Zürich*, 1876, p. 36; Kaufmann, M.: *Arch. f. d. ges. Physiol.*, cix, p. 443, 1905; Rona and Müller: *Zeitschr. f. physiol. Chem.*, l, p. 263, 1906; Abderhalden: *ibid.*, lxxvii, p. 22, 1912.

EXPERIMENTAL PART.

Gliadin and lysine.

That gliadin is adequate to supply the needs of the animal organism in respect to its nitrogenous requirements for maintenance over very long periods of time has been convincingly shown in our own experience.[12] Henriques[13] succeeded in maintaining the body-weight of rats unchanged for nearly a month on gliadin food, and claimed that if the protein is fed in abundance not only nitrogenous equilibrium, but even a retention of nitrogen in the body may result. We have found, however, that the method of collecting urine as devised by Henriques is attended by losses which leave the quantitative aspects of the subject uncertain. The failure to collect all of the nitrogen excreted would thus *appear* in favor of the storage of nitrogen. Growth was not reported by Henriques, and he failed to recognize the importance of the missing lysine.

The ready digestibility of gliadin is attested by numerous investigators.[14] Abderhalden was unable to replace protein successfully by the products of the complete digestion of gliadin, even when the missing lysine was supplied in abundance.[15] He remarks that the proportion in which the various amino-acids are present is probably too little adapted for reconstruction of tissue.[16]

We have succeeded in promoting growth at a normal rate when a maintenance ration containing gliadin as the sole protein was supplemented with lysine. The success of the present series of feeding experiments has been greatly enhanced by the discovery that butter-fat promotes the growth of rats kept on the diets of isolated foodstuffs and "protein-free milk" which we have been accustomed to employ for several years.[17] Possible failures owing

[12] Cf. Osborne and Mendel: The Rôle of Gliadin in Nutrition, this *Journal*, xii, pp. 473–510, 1912.

[13] Henriques: Lässt sich durch Fütterung mit Zein oder Gliadin als einziger stickstoffhaltiger Substanz das Stickstoffgleichgewicht herstellen? *Zeitschr. f. physiol. Chem.*, lx, p. 105, 1909.

[14] Cf. Mendel and Fine: The Utilization of Proteins of Wheat, this *Journal*, x, p. 303, 1911.

[15] Cf. Abderhalden: *Synthese der Zellbausteine in Pflanze und Tier*, Berlin, 1912, p. 85.

[16] Abderhalden: *Zeitschr. f. physiol. Chem.*, lxxvii, p. 29, 1912.

[17] Osborne and Mendel: this *Journal*, xv, pp. 311–326, 1913; *ibid.*, xvi, pp. 423–437, 1913.

to the lack of suitable, as yet unknown accessory factors have been averted in this way.

The demonstration that the addition of lysine to the gliadin food serves to render this protein of wheat entirely adequate for the nitrogenous needs of growth is shown in Chart I, Rat 1113, in the appendix, in which the surprising effect of this amino-acid addition is in strong contrast with the mere maintenance effect of the diet without the lysine. The diets here referred to had the following composition:

Gliadin food mixtures.
(See Chart I.)

	WITHOUT LYSINE	WITH LYSINE[18]
	grams	*grams*
Gliadin[19]	18.0	17.46
Lysine	none	0.54
Protein-free milk	28.0	28.00
Starch	24.0	21.34
Butter-fat	18.0	18.00
Lard	12.0	14.00

How promptly the growing organism responds to the presence or lack of a nutritive unit indispensable for growth is even more strikingly shown in Chart I, Rats 1846, 1844, and 1850, in which the alternate addition and withdrawal of the lysine is followed by an immediate and unmistakable response in the character of the curve of body-weight.

We believe that these feeding trials, in conjunction with our demonstration of the almost complete cessation of growth on diets containing only lysine-free proteins, furnish the first and only conclusive demonstration that *lysine is indispensable for the func-*

[18] In preparing this food pure crystallized lysine dichloride was mixed with enough sodium carbonate to neutralize the hydrochloric acid combined with the lysine. This was then mixed with the finely ground gliadin, starch and protein-free milk. After thoroughly mixing all of these they were stirred into the melted lard and butter-fat. The final and complete mixing of all these ingredients with the fat was effected by passing the whole several times through a meat chopper.

[19] Regarding the content of lysine in our gliadin see Osborne and Mendel: The Rôle of Gliadin in Nutrition, this *Journal*, xii, pp. 473–510, 1912; also Osborne and Leavenworth: Do Gliadin and Zein Yield Lysine on Hydrolysis? *Ibid*, xiv, pp. 481–487, 1913.

tions of growth. They are supplemented by further evidence of the same sort in which the necessity for the same amino-acid is brought out in connection with the zein of maize, a protein likewise devoid of lysine (see Charts V and VI).

Lysine in other proteins in relation to growth.

The facts here established make it clear that, at least in so far as nutrition in growth is concerned, the normal construction of new tissues is limited by the factor of the supply of lysine. In the light of this, little is gained by emphasizing the quantitative aspects of the protein needs in growth as conspicuously as Rubner and others have done, unless the qualitative character of the protein available is kept clearly in mind. No amount of energy or protein, however abundant, has induced growth of our animals in the absence of lysine. The animal organism apparently cannot synthesize lysine, which is evidently not essential for maintenance in the sense of preservation of body-weight, though it is of course impossible to say that when this amino-acid is missing all functions are normally carried out. That the tissues either form a typical protoplasmic product, or none at all, now seems to be axiomatic in physiology. We may therefore reasonably assume that the growth of rats on our gliadin + lysine food represents the construction of typical tissue substance. It is obvious, furthermore, that the possibility of growth must be limited, among other things, by the amount of lysine available. How widely proteins differ in their content thereof, is shown by the following data taken from the best analyses at hand:

Lysine in proteins.[20]

	per cent		*per cent*
Lactalbumin	8.10	Glutelin, maize	2.93
Halibut muscle	7.45	Glutenin, wheat	1.92
Ox muscle	7.59	Edestin, hemp-seed	1.65
Casein, cow's milk	7.61	Amandin, almond	0.72
Vitellin, egg-yolk	4.81	Gliadin, wheat	0.16
Crystallized albumin, hen's egg	3.76	Hordein, barley	0.00
Legumin, pea	4.98	Zein, maize	0.00
Phaseolin, kidney bean	4.58		

[20] These data have been obtained by Kossel and Kutscher's method. The recent investigations of Van Slyke and his associates indicate that these figures are probably a little low. Cf. this *Journal*, xvi, p. 531, 1914.

It is a teleologically interesting fact brought out by the foregoing figures that those proteins, like casein, lactalbumin, and egg vitellin, which are in nature concerned with the growth of animals, all show a relatively high content of lysine. Further presumptive evidence in respect to the rôle of lysine here portrayed is furnished by experiments in which the relative efficiency of different proteins in transforming a diet that is inadequate for growth into one that promotes growth is clearly brought out. Zein, like gliadin, is devoid of lysine-yielding complexes and cannot promote growth. It can be made to suit the needs of growth better, as we shall see later, if all of its amino-acid deficiencies are suitably made good. When other proteins are furnished in addition to zein in the diet, the proportions required to render the ration satisfactory for growth are unlike with respect to the quantities necessary to permit the animal to make normal growth with a minimum of the supplementary protein. In other words, the zein diet may be rendered efficient for normal growth by replacing a part of the zein with other proteins containing the amino-acids which zein lacks. The minimal proportion thus required is not the same for each protein, but is determined by the proportion of that amino-acid, thus supplied, which is present in the least amount in the added protein. Lactalbumin and edestin, for example, are in strong contrast in respect to their content of lysine (see table p. 334). Either of these proteins, like certain others that we have tried, will supplement a zein ration so as to permit adequate growth. The proportion necessary for this purpose is, however, different for the two proteins in accord with their unlike lysine yield. Thus when the lysine-rich lactalbumin is used, a 25 per cent replacement suffices for perfect growth, whereas in the case of edestin, low in lysine, a similar substitution results in no increment of weight. In order to obtain growth approximating the normal it is necessary to replace one-quarter of the zein of our standard ration with lactalbumin, or three-quarters with edestin (Chart II). The quantitative insufficiency of smaller proportions of edestin is shown in Chart III. That the lysine is the controlling factor is demonstrated by Rats 1807, 1799 and 1531 in Chart III which also show the effect of addition of lysine to an inadequate proportion of edestin. Such experiments have been duplicated repeatedly, and speak plainly for the dominating importance of lysine in the synthetic nutrition of growth.

Zein and amino-acids.

Zein presents even more striking differences than gliadin in its amino-acid make-up when compared with the other proteins commonly present in foods. The greatest interest has centered around the entire absence of glycocoll, tryptophane and lysine; for feeding experiments with zein were expected to shed light on the important question of amino-acid synthesis by the animal. The deviations of zein from other proteins which we have used extensively for feeding are further emphasized below:

Comparative composition of proteins.

AMINO-ACIDS	ZEIN (MAIZE)	GLIADIN (WHEAT)	CASEIN (MILK)	LACTALBUMIN (MILK)	EDESTIN (HEMP-SEED)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Glycocoll	0.00	0.00	0.00	0.00	3.80
Alanine	13.39	2.00	1.50	2.50	3.60
Valine	1.88	3.34	7.20	0.90	6.20
Leucine	19.55	6.62	9.35	19.40	14.50
Proline	9.04	13.22	6.70	4.00	4.10
Phenylalanine	6.55	2.35	3.20	2.40	3.09
Aspartic acid	1.71	0.58	1.39	1.00	4.50
Glutaminic acid	26.17	43.66	15.55	10.10	18.74
Serine	1.02	0.13	0.50	?	0.33
Tyrosine	3.55	1.50	4.50	2.20	2.13
Cystine	?	0.45	?	?	1.00
Histidine	0.82	1.49	2.50	1.53	2.19
Arginine	1.55	3.16	3.81	3.01	14.17
Lysine	0.00	?	7.61	8.10	1.65
Tryptophane, about	0.00	1.00	1.50	+	+
Ammonia	3.64	5.22	1.61	1.32	2.28
	88.87	84.72	66.92	56.46	82.28

The failure of animals to grow or even be maintained when zein is the sole form of nitrogenous food-intake for any considerable period has been widely recognized. Feeding experiments conducted with rats by Henriques[21] show in some protocols surprising-

[21] Henriques: *Zeitschr. f. physiol. Chem.*, lx, p. 108, 1909.

ly small decline in body-weight during the relatively short periods of two weeks, or less, within which they were continued. This investigator contents himself with the conclusion that nitrogenous equilibrium cannot be obtained with zein as the sole source of nitrogen in the diet. The digestibility of zein has frequently been found to be quite poor. This seems, however, to be in good measure due to the physical character of the isolated zein which tends to assume a hard, resistant form, even when finely ground. "Coefficients of digestibility" ranging from 50 to 90 per cent are reported for corn proteins, but there are few data derived from experiments conducted under ideal conditions.[22] In our own work we have long taken the precaution to hydrate the zein by incorporating a little water in the food; and we cannot charge the poor nutritive results to undue lack of absorption of the digestion derivatives of zein.

The inability of zein food to maintain animals adequately is attested by the experience of Willcock and Hopkins.[23] In experiments with mice they found that zein "has no power whatever of maintaining growth in the young animal; loss of weight begins the moment it forms the sole nitrogenous supply." They further remark:

The addition of the missing tryptophane group has, it is also clear, no power to convert such loss into equilibrium or gain: a fact possibly due to other deficiencies in the zein molecule, such as the absence of lysine, or the lack of some other amino-acid not yet observed. There was no close relationship in our experiments between the loss of weight and the length of survival period. In many individual cases the mice upon tryptophane lost a considerably larger percentage of their weight before death than, on the average, did those without it. Such differences may be largely due to differences in the nutritional condition of individuals at the outset, but the results appear to show that death was not determined by a critical percentage loss. On the other hand the figures show that, on the average, the loss of body-weight was slower with tryptophane than without it. But this result might well be expected, even if the tryptophane administered undergoes utilization without directly contributing to tissue formation or structural maintenance. If it serves as a basis for the elaboration of a substance absolutely necessary for life—something, for instance, of an importance equal to that of adrenaline—then, in starvation, or when it is

[22] Cf. Mendel and Fine: this *Journal*, x, p. 345, 1911.
[23] Willcock and Hopkins: *Journ. of Physiol.*, xxxv, pp. 88-103, 1906.

absent from the diet, a supply is likely to be maintained from the tissue-proteids; the demand for it would become one of the factors determining tissue breakdown. In the case of young animals which directly benefit from the addition of a protein constituent, otherwise absent from their diet, to the extent of a well nigh doubled life, and marked improvement in general condition, but at the same time steadily lose, instead of gaining, weight, the utilization of the constituent would seem to be of some direct and specific nature.

McCollum[24] appears to have had somewhat better success in feeding pigs with zein-food. He maintained an animal unchanged in body-weight for three weeks on zein as the sole protein (see McCollum, Table III, p. 225) and remarks: "All previous efforts to induce growth in animals by feeding zein as the sole source of protein have failed entirely, but the pig appears to be exceptionally efficient in the utilization of foodstuffs, so it seemed possible that more favorable results might be met with in the case of zein."

We have now accumulated the results of a large experience in feeding zein to both adult and growing rats with the uniform consequence of decline, when this protein forms the sole nitrogenous component of the dietary. Reference to Chart IV will suffice to show the general character of numerous trials during intervals of several years with outcomes differing only in the greater or lesser rapidity of the decline. We may point out that our zein has always been purified with care, so that contamination with other corn proteins has been avoided. The importance of this may be realized in view of the fact that small additions of these are sufficient to prevent decline. Furthermore we have not attempted to induce our animals at the same time to consume undue amounts of non-nitrogenous foods, as can so effectively be done in the case of the pig, with the result of preventing fall in body-weight for surprisingly long periods even in the absence of any protein intake.[25]

The best evidence of the unique *rôle of tryptophane* and its indispensability to nutrition in preserving maintenance in the organism, is shown by comparing the curves of body-weight of rats furnished food containing zein + tryptophane with those of rats on foods containing zein without this addition. (See Charts IV

[24] McCollum, E. V.: *Amer. Jour. of Physiol.*, xxix, pp. 215–236, 1911.

[25] Cf. McCollum, E. V.: *Amer. Journ. of Physiol.*, xxix, pp. 215–237; Grafe, E.: *Zeitschr. f. physiol. Chem.*, lxxxviii, pp. 389–424, 1913.

and V.) It is to be noted that those rats which had tryptophane declined slowly except 1892 and 1895 which were almost perfectly maintained for more than ninety-four and sixty-six days respectively. These two rats were supplied with butter-fat in place of a part of the lard in their diet. The beneficial effects of this addition were not known at the time the other experiments were in progress. That this factor had no favorable influence when zein alone was fed is shown in Chart IV by Rats 684, 1773, 1890, and 710 which declined as rapidly as the others, although their food contained 18 per cent of butter-fat.

In the comparable experiments with mice which Miss Wheeler[26] conducted at our suggestion, the beneficial effect of tryptophane additions to zein food was more pronounced than appears in the records of Willcock and Hopkins. Mice fed by the latter on zein died before the twelfth day; those fed with zein + tryptophane were alive and active on the sixteenth day when the experiment was terminated, but they had lost weight. In Miss Wheeler's experiments mice fed with zein lost on the average one-third of their weight in twenty-five days; while two mice which had an addition of tryptophane equal to 3 per cent of the zein fed, lost only about one-fifth of their original weight by the fiftieth day. It is of interest to note that in Miss Wheeler's experiments gelatin was far less effective in its nutritive powers than was zein; and that with a diet containing equal parts of zein and casein satisfactory maintenance of mice was secured.

The lack of tryptophane can also be made good, as might be expected, by supplementing the zein ration with proteins which do contain this lacking amino-acid. Thus even gliadin stopped the decline (see Chart I, Rat 1113). The relative efficiency of different proteins in preventing the failure with zein apparently depends to a dominant degree, in so far as maintenance is concerned, on their comparative yield of tryptophane.

Where growth is involved in addition to maintenance, the lysine factor, as well as others, not yet more accurately ascertained, must also be taken into account. Here, then, is evidence of the relative economy of different proteins in maintenance, based upon the content of one or more of the amino-acids essential for the proper func-

[26] Wheeler, Ruth: Feeding Experiments with Mice, *Journ. of Exp. Zoöl-ogy*, xv, pp. 209–223, 1913.

tioning of the organism (in so-called maintenance) or for new tissue construction (in growth). *Obviously the relative values of the different proteins in nutrition are based upon their content of those special amino-acids which cannot be synthesized in the animal body and which are indispensable for certain distinct, as yet not clearly defined processes which we express as maintenance or repair.*

This is a viewpoint somewhat different from the general hypothesis of Abderhalden, that the greater the similarity of the molecule of food protein to that of the body proteins, the greater will be the food value to the animal. It likewise differs from the conclusions drawn by Michaud[27] in his attempt to substantiate the foregoing. He states that the protein minimum is most readily attained if the protein of the same species (körpereigenes Eiweiss) is fed, and that more is required whenever protein of a different amino-acid make-up is fed. The implication, of course, is that the protein minimum represents a total destruction of protein which can best be made good by supplying all of the lost amino-acids in precisely the proportions in which they existed in the catabolized body protein. Bearing in mind the possibility, developed in our introductory remarks, that the destruction in maintenance (apart from any need of energy not supplied by non-protein sources) may represent chiefly a device for getting one or more amino-acids essential for some regulatory, or similar function, rather than *all* that are needed for reconstruction of tissue destroyed, Michaud's findings can be explained quite as well upon this hypothesis. We need merely point out that dog-meat, casein, edestin, "glidin" and serum proteins probably differ greatly in their yield of the indispensable amino-acids. When therefore an animal is put upon short protein rations it may well be that those richest in tryptophane, for example, will best fill the minimum maintenance requirement. Muscle tissue which is richer than edestin in lysine, as happened in Michaud's experiments, ought to prove more economical. It is not improbable that the relatively successful records of dog's meat in Michaud's experiments can be equalled with other proteins, like lactalbumin, which can scarcely be called "körpereigenes Eiweiss." The problem of protein minimum needs to be approached from new standpoints.

[27] Michaud: *Zeitschr. f. physiol. Chem.*, lix, p. 405, 1909.

With the indispensability of tryptophane for maintenance, and of lysine for growth, thus emphasized, we may expect that the addition of *both* of these amino-acids to zein food will result in growth. This expectation has been fulfilled by the experiments planned (see Chart VI). The respective parts played by the two amino-acids is here clearly brought out. *These are*, we believe, *the first successful attempts to grow animals on a diet in which zein forms the sole protein.* If we compare Charts VII, VIII and III which show the relative effect of replacing one-fourth of the zein with lactalbumin, casein, and edestin respectively it will be seen that this small addition of lactalbumin has furnished all the factors required for normal growth. This protein mixture therefore undoubtedly contains sufficient tryptophane and lysine to satisfy the normal requirements of the growing animal. Since casein yields nearly as much lysine as does lactalbumin, we assumed that the failure of an equal addition of casein was due to a relative deficiency in tryptophane. That this asumption was correct is shown by Rats 1808 and 1809 in Chart VIII; the addition of a small amount of tryptophane to the previously inefficient zein + casein diet at once resulted in rapid growth. Similarly we assumed that the failure to grow on the comparable zein + edestin food was caused by the relatively small amount of lysine yielded by edestin. Here, too, the correctness of our assumption is demonstrated by Rats 1807 and 1799 (Chart III) in which the addition of lysine rendered the food mixture adequate for growth at a normal rate. These experiments are further of chemical interest in indicating, as the result of this biological test, that edestin is richer in tryptophane than casein—a fact hitherto unappreciated.

The growth of rats on a food of zein + lysine + tryptophane has not always been as rapid and prolonged as we might expect. We are by no means prepared to maintain that the final solution of the proportion of amino-acids requisite for the growth of rats has been determined. Newer trials may indicate the desirability of increasing the proportion of arginine present in zein foods; and still other adjustments may be required to promote ideal growth in this or different species. The way to successful investigation has been opened.

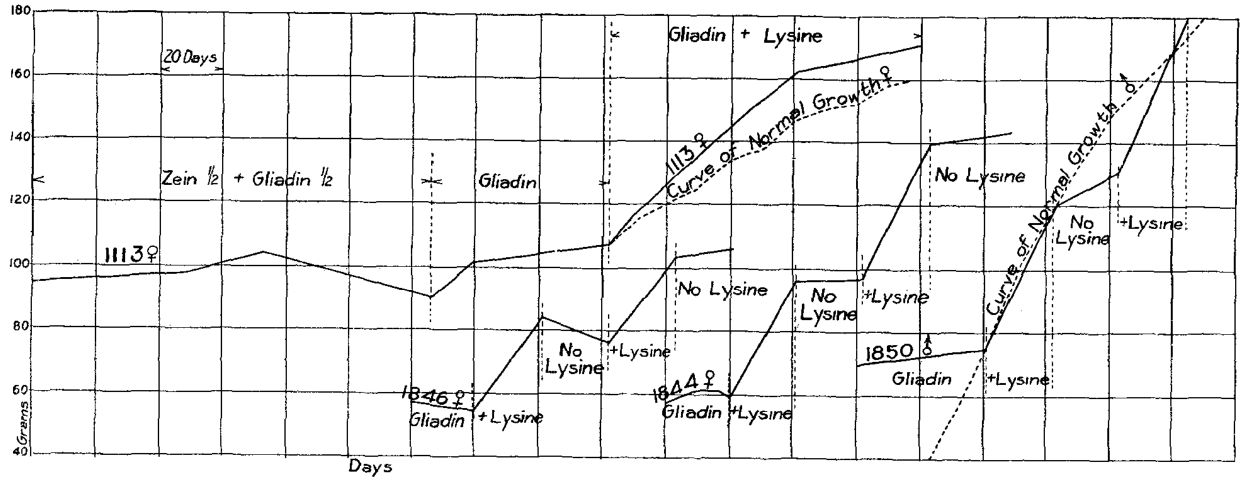

CHART I. INDISPENSABILITY OF LYSINE FOR GROWTH. This chart shows the *failure to grow on diets containing gliadin* as the sole protein; the immediate resumption of *growth when lysine equivalent to 3 per cent of the protein is added to the food;* and the equally prompt *cessation of growth when the addition of lysine is stopped.*

Animals cannot be maintained on zein food alone. Inasmuch as gliadin does not yield more than insignificant amounts of lysine, practically no growth is made on foods containing gliadin as the sole protein. A food containing equal parts of zein and gliadin serves to maintain body-weight. See Rat 1113. This indicates that not all of the amino-acids yielded by proteins are necessary for maintenance; otherwise we would expect destruction of tissue and fall of body-weight in the experiment just cited.

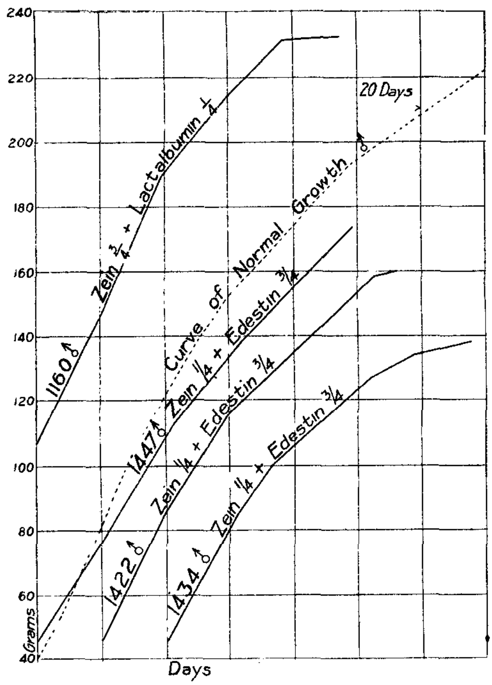

Chart II. Relative Proportions of Proteins Yielding Different Amounts of Lysine Which Promote Normal Growth when They Replace a Part of the Zein. Rat 1160 shows growth at a normal rate when *one-fourth of the zein in the food is replaced by lactalbumin* which yields over 8 per cent of lysine. Rats 1422, 1434, 1447 show growth at a normal rate when *three-fourths of the zein in the food is replaced by edestin* which yields 1.65 per cent of lysine. That smaller additions of edestin fail to promote normal growth is shown in Chart III.

The composition of the foods used was as follows:

	Rat 1160	Rats 1422, 1434, 1447.
	grams	*grams*
Zein	13.5	4.5
Lactalbumin	4.5	0.0
Edestin	0.0	13.5
Protein-free milk	28.0	28.0
Starch	27.0	26.0
Lard	27.0	28.0
Water	12 cc.	4 cc.

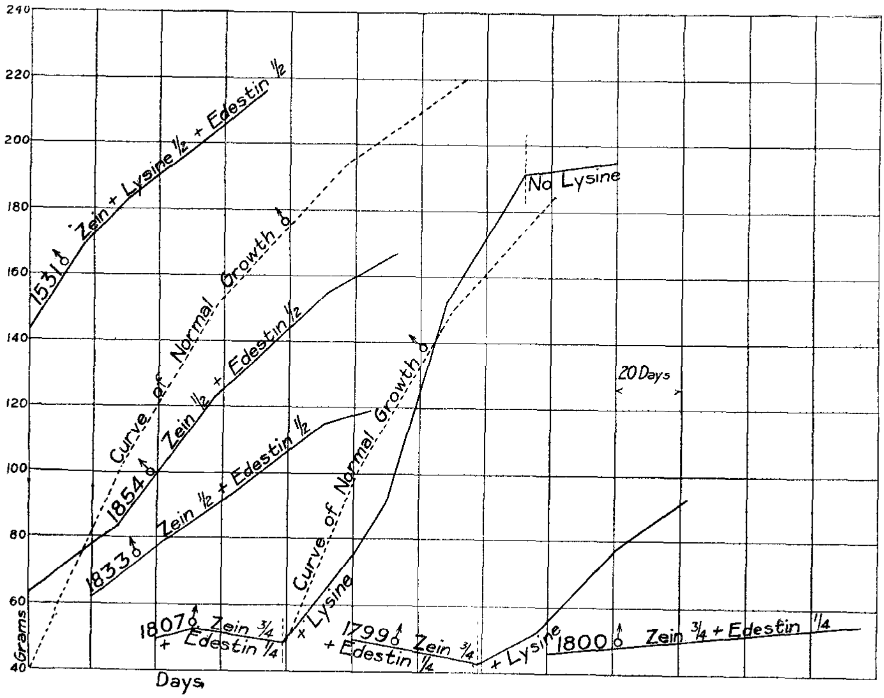

Chart III. Rôle of Lysine in Growth. Inasmuch as edestin yields relatively little lysine (1.65 per cent) considerable additions of this protein to our zein food are necessary to produce growth. Thus, *when one-quarter of the zein is replaced by edestin no growth is obtained*, unless lysine as such is also added, whereupon rapid growth results. See Rats 1799, 1800, 1807. *When one-half of the zein is replaced, better growth obtains.* See Rats 1833, 1854. When lysine is added to a food containing equal parts of zein and edestin, practically normal growth results. See Rat 1531. *Normal growth is obtained by a three-quarter replacement with edestin.* (See Chart II, Rats 1422, 1434, 1447.)

The foods consisted of:

	Rats 1833, 1854.	Rats 1799*, 1800, 1807*.	Rat 1531.
	grams	*grams*	*grams*
Zein	9.0	13.5	8.70
Edestin	9.0	4.5	9.00
Lysine	0.0	0.0	0.54
Protein-free milk	28.0	28.0	28.00
Starch	24.5	25.7	24.50
Butter-fat	18.0	18.0	18.00
Lard	11.5	10.3	11.50
Water	7 cc.	12 cc.	7 cc.

* These rats received food containing an addition of lysine equivalent to 3 per cent of the zein, as indicated on the chart.

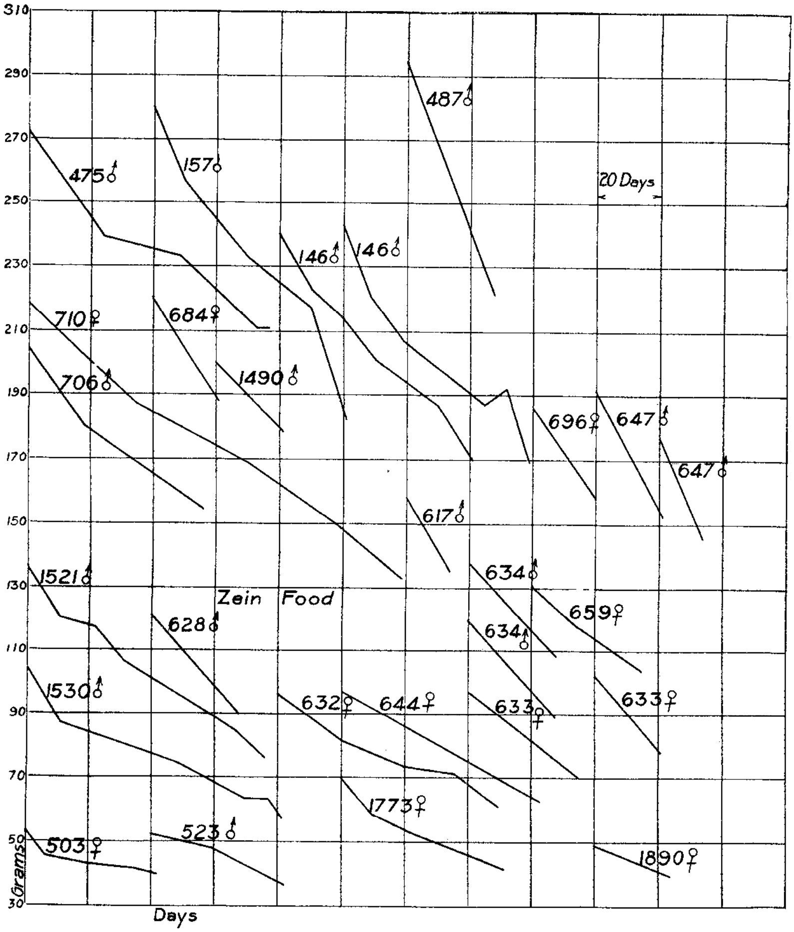

CHART IV. EXPERIMENTS WITH ZEIN. *Neither growth nor maintenance can be secured when zein is the sole protein in the dietary.*

The foods for this series varied somewhat in details of composition, but contained approximately:

	grams
Zein	18
Protein-free milk	28
Starch	27
Butter-fat Lard	27
Water	15 cc.

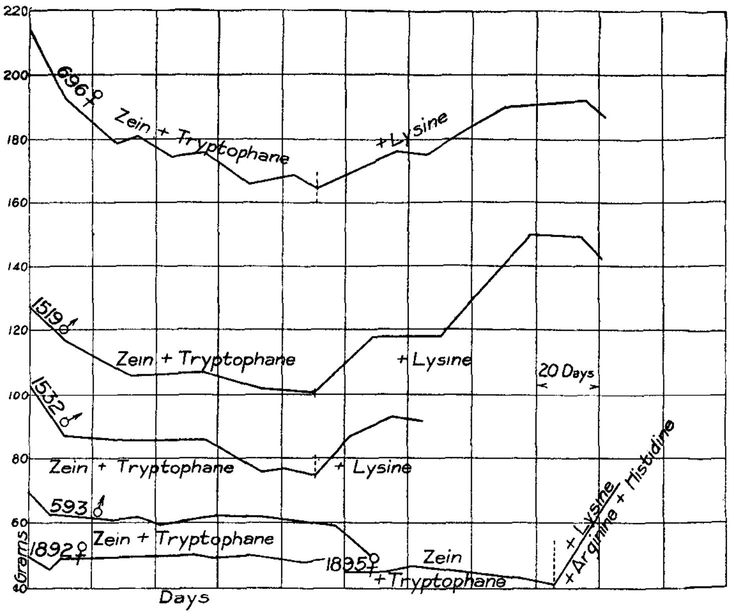

CHART V. INDISPENSABILITY OF TRYPTOPHANE FOR MAINTENANCE IN NUTRITION. These experiments should be contrasted with the failure of maintenance on zein-food alone, shown in Chart IV. Rats 696, 1519, 1532 show comparatively slow decline and *tendency to maintenance when tryptophane replaced 3 per cent of the zein in the food.* Rats 593, 1892, 1895 were *almost perfectly maintained* on similar foods for comparatively long periods. Rats 1892, 1895 received butter-fat in place of a part of the lard in their diet. The effect of further *additions of lysine* equivalent to 3 per cent of the zein, in *promoting recovery or growth* is seen in Rats 696, 1519, 1532, 1895. Further instances may be seen in Chart VI.

The foods contained·

	Rats 696* 1519,* 1532,* 593.	Rats 1892, 1895.*
	grams	*grams*
Zein	17.46	17.46
Tryptophane	0.54	0.54
Protein-free milk	28.00	28.00
Starch	27.00	27.00
Butter-fat	0.00	18.00
Lard	27.00	9.00
Water	15 cc.	15 cc.

* Lysine equal to 3 per cent of the zein was added to the diet during part of the time as indicated in the chart. Rat 1895 also received an addition of arginine equivalent to 1.5 per cent and histidine equivalent to 1 per cent of the zein.

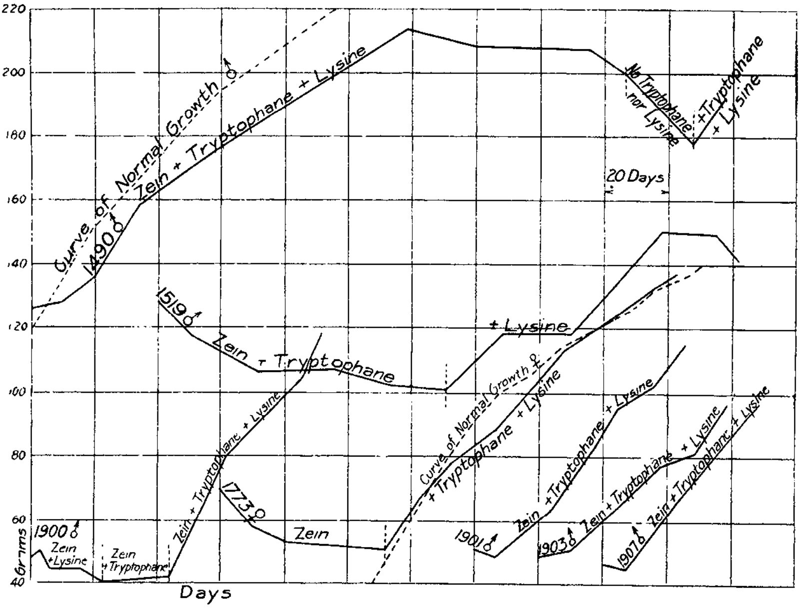

CHART VI. GROWTH ON FOODS CONTAINING ZEIN+TRYPTOPHANE+LYSINE.

The food contained:

	grams
Zein	16.92
Tryptophane	0.54
Lysine	0.54
Protein-free milk	28.00
Starch	27.00
Butter-fat	18.00
Lard	9.00
Water	15 cc.

The *growth* obtained on this diet may be contrasted with *maintenance without growth* in the absence of the lysine (see Chart V) and *failure to be maintained* in the absence of both lysine and tryptophane (Chart IV), thus demonstrating the rôle of these amino-acids in growth and maintenance respectively. That *lysine cannot replace tryptophane in maintenance* is shown by Rat 1900.

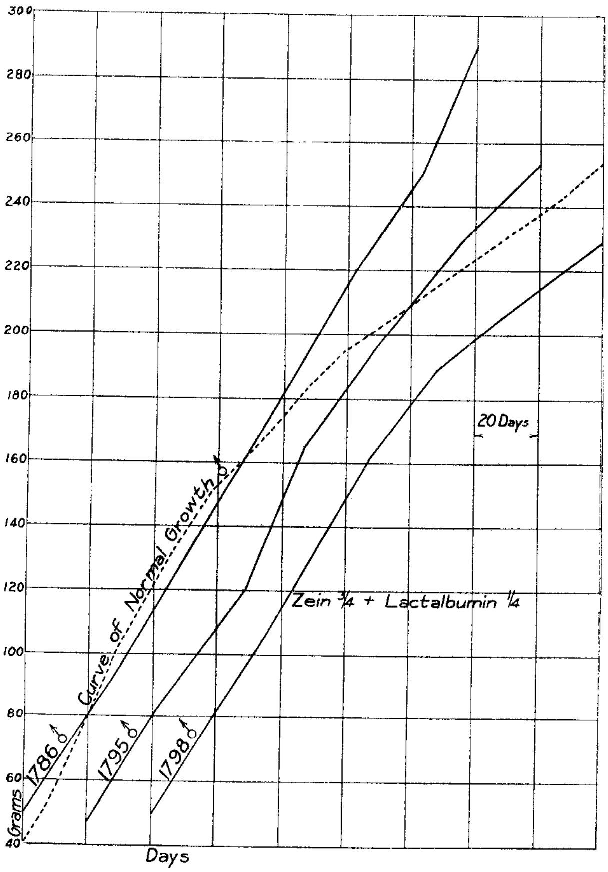

CHART VII. GROWTH ON FOOD IN WHICH ONE-QUARTER OF THE ZEIN IS REPLACED BY LACTALBUMIN.

The food consisted of:

	grams
Zein	13.5
Lactalbumin	4.5
Protein-free milk	28.0
Starch	27.3
Butter-fat	18.0
Lard	8.7
Water	12 cc.

The proportion of lactalbumin here represented obviously furnished sufficient lysine and tryptophane to permit growth at an approximately normal rate. Compare failures with corresponding additions of casein (Chart VIII) or of edestin (Chart III).

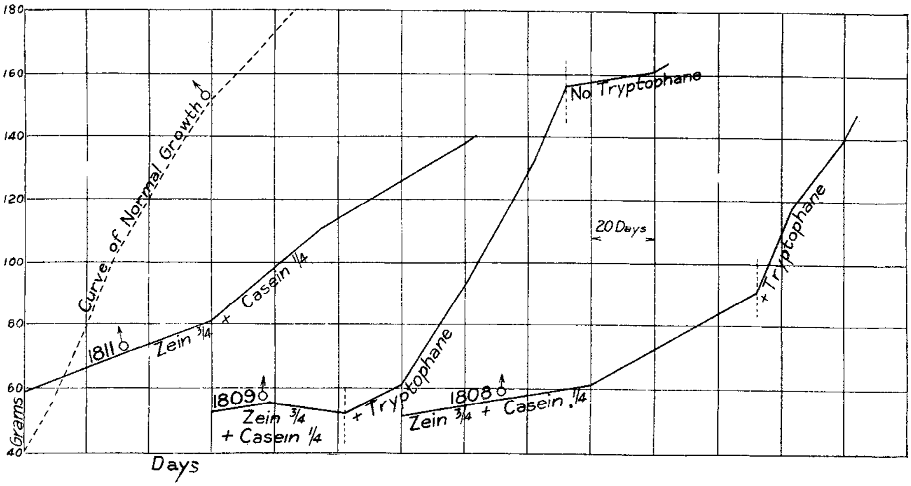

CHART VIII. EFFECT ON GROWTH OF REPLACING ONE-QUARTER OF THE ZEIN IN OUR FOODS WITH CASEIN.

The foods contained (prior to the addition of tryptophane equivalent to 3 per cent of the zein):

	grams
Zein	13.5
Casein	4.5
Protein-free milk	28.0
Starch	28.5
Butter-fat	18.0
Lard	8.5
Water	12 cc.

The *inadequacy of this mixture for satisfactory growth* must be compared with the success attending the use of the same proportion of lactalbumin (Chart VII). That this failure of the same proportion of casein to supplement the imperfect zein is due to its relative deficiency in tryptophane is demonstrated by the prompt renewal of growth when this amino-acid is added, and the cessation of growth when it is again omitted.

AMINO-ACIDS IN NUTRITION AND GROWTH

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