NUTRITIVE PROPERTIES OF PROTEINS OF THE MAIZE KERNEL.[1]

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The enormous production of maize in this country makes a knowledge of every factor concerning its nutritive value not only of scientific interest, but also of great economic importance. Experience in feeding the seeds of maize has led to the belief that, in comparison with many other available food stuffs, it is in some respects inadequate. To account for the nutritive deficiency various suggestions have been made, such as the low content of protein; the small percentage of ash; the relatively small proportion of calcium and large proportion of magnesium in the ash; and, recently, it has appeared possible that the peculiar chemical constitution of zein, the chief protein of this seed, may, in whole or in part, furnish an explanation. We have accordingly undertaken to determine the relative nutritive value of zein when fed in combination with other proteins of maize as well as of some other seeds and of milk and give in the following pages the results thus far obtained.

In order that the data here presented may be better understood in their relation to the practical use of maize products as food for animals or men it is necessary to call attention to the fact that this seed contains several distinct proteins which differ from one another in their solubilities and chemical constitution. The structural and nutritive deficiencies of zein are supplemented by these other proteins to a greater or less extent, and consequently

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

the statements relating to zein made in the following pages must not be understood to apply to the combination of proteins existing in the entire seed.

The most abundant protein of the maize kernel is zein which is soluble in relatively strong alcohol or dilute aqueous alkaline solutions, but insoluble in water or in solutions of neutral inorganic salts. The next most abundant is maize glutelin, insoluble in water, saline solutions or alcohol, but readily soluble in dilute sodium or potassium hydroxide solution. These two proteins contain about 72 per cent of the nitrogen of the entire maize kernel. About 22 per cent of the total nitrogen of the seed is soluble in saline solutions, such as 10 per cent sodium chloride solution, and probably belongs mostly to protein substances. From such extracts of corn meal Chittenden and Osborne[2] isolated three globulins, an albumin and a small amount of protein having the properties of proteose. Since the proportion of each of these is small, nothing has been learned of them beyond their ultimate composition and a few facts concerning their solubility. We have no information whatever as to their amino-acid make-up.

All of the foregoing proteins of the maize kernel are soluble in dilute alkaline solutions, but such solutions do not extract all of the nitrogen from even very finely ground corn meal. Whether any, or all, of this undissolved nitrogen belongs to proteins enclosed in unruptured cells or to non-protein substances insoluble in the solvents mentioned is unknown. Owing to the difficulties encountered in extracting these different types of protein, and in separating them completely from one another, no accurate statement can be made of the proportions in which they exist in the maize kernel. Some years ago one of us attempted to estimate the proportions of these several proteins in a sample of yellow corn which contained 1.54 per cent of nitrogen. The results of this attempt can be summarized as follows:

	Per cent
Globulins, albumins, and "proteoses"	0.45
Zein	5.00
Maize glutelin	3.15
Insoluble N × 6.25	1.03
	9.63

[2] Chittenden and Osborne: *Amer. Chem. Journ.*, xiii, pp. 453–468, 529–552, 1891; and xiv, pp. 20–44, 1892.

Further experience has shown that the aggregate amount of the proteins soluble in dilute saline solutions, *i.e.*, of the globulins, albumins, and "proteoses," is probably much greater than indicated by these figures which were based on the weights of preparations of these proteins actually isolated from the seed.

A more recent attempt, in which we were assisted by L. I. Holdredge,[3] showed that about one-fifth of the nitrogen of a sample of white corn, containing 2.33 per cent thereof, was soluble in 10 per cent potassium chloride solution. Since most of this nitrogen undoubtedly belongs to protein we feel convinced that the proportion of the proteins soluble in saline solutions in our earlier analyses was stated too low. The following table shows the proportion of nitrogen found in the different parts of the seed.

White corn dried at 110°: N = *2.33 per cent.*

	PER CENT OF CORN	CONTAINING N	
		Per cent of part	Per cent of corn
Hulls and tipcaps	8.5	1.52	0.12*
Embryo	11.0	3.42	0.38
Endosperm	80.5	2.28	1.84
	100.0		2.34

* The hulls alone contained 0.03 per cent N.

N *extracted in per cent of total* N.

	WHOLE CORN	ENDO-SPERM	EMBRYO	HULLS AND TIPCAPS
N soluble in 10 per cent KCl solution	22.0	7.8	77.2	not determined
N soluble in 90 per cent alcohol	41.0	50.0	2.0	
N soluble in 0.2 per cent KOH solution	31.0	38.2	0.6	
N insoluble and loss	6.0	4.0	20.2	

[3] Not published.

N extracted in per cent of corn and its parts.

	WHOLE CORN	ENDO-SPERM	EMBRYO	HULLS AND TIPCAPS
N soluble in 10 per cent KCl solution	0.51	0.14	0.29	
N soluble in 90 per cent alcohol....	0.96	0.92	0.01	
N soluble in 0.2 per cent KOH solution....	0.72	0.70	trace	
N insoluble and loss....	0.14	0.08	0.08	
Total N in corn....	2.33	1.84	0.38	0.12

N in: Endosperm, 1.84; Embryo, 0.38; Hulls and tipcaps, 0.12 = 2.34
N in the whole corn.... 2.33

	ENDOSPERM + EMBRYO		FOUND IN WHOLE CORN
N soluble in 10 per cent KCl solution...	0.14	0.29 = 0.43	0.51
N soluble in 90 per cent alcohol	0.92	0.01 = 0.93	0.96
N soluble in 0.2 per cent KOH solution.	0.70	trace = 0.70	0.72
N insoluble and loss	0.08	0.08 = 0.16	0.14
N in hulls and tipcaps		= 0.12	
Total N....		2.34	2.33

Assuming, as is probably nearly correct, that all of the nitrogen of the corn belongs to protein containing 16 per cent N, we have the following:

	PER CENT OF CORN	PER CENT OF PROTEIN
Globulins + albumins + "proteoses"....	3.19	21.9
Zein....	6.00	41.4
Maize glutelin....	4.50	30.8
Insoluble in alkali....	0.88	5.9
	14.57	100.0

These figures cannot be accepted as exactly representing the actual proportions of nitrogen soluble in the various solvents, for many, as yet unsurmountable difficulties render complete extractions and accurate determinations impossible. We believe, however, that they give a fairly good idea of the relative proportions of the different types of protein, and must serve until more exact data can be secured.

In so far as they cover the same ground, the preceding data agree well with those given by Hopkins, Smith and East,[4] who found a similar distribution of nitrogen among these different parts of a sample of high nitrogen corn. From these figures we can conclude that a little less than one-half of the protein substance in the entire maize kernel is zein. Since maize glutelin, the next most abundant protein, has been shown to yield all of the amino-acids which zein lacks, and as it is probable that the remaining proteins likewise yield them, the amino-acid deficiencies of zein are thus more or less supplemented when the entire seed is fed.

In the feeding experiments described in this paper a product was used which we have designated "corn gluten." This was kindly prepared for us under the direction of Mr. H. C. Humphrey of the Corn Products Refining Company. In manufacturing corn starch the seeds are softened in water containing sulphurous acid and the contents of the cells of the endosperm are separated from the other parts of the seed by grinding under water in suitable mills and straining out the hulls, tipcaps and embryos on sieves. The starch grains and suspended protein thus set free from the cells of the endosperm, after passing through the sieve, are carried slowly by the flowing water over long troughs, upon the bottom of which nearly all the starch is deposited so that the material passing out of the trough consists of nearly one-half endosperm protein and one-half starch, endosperm cell walls and other insoluble carbohydrates, and some oil. This material was filter-pressed, dried at a low temperature and sent to us as a source from which to obtain zein and maize glutelin.

The following figures show the results of our partial analysis of this preparation of "corn gluten."

	Per cent of the "corn gluten" dried at 110°
Inorganic matter	0.15
Total nitrogen	7.19
Ether-soluble matter	5.25
N soluble in hot alcohol	4.99
N soluble in hot alcohol × 6.25 = zein	31.25
Total N minus alcohol-soluble N = 2.20 × 6.25 = maize glutelin	13.75
Total protein	45.00

[4] Hopkins, Smith and East: Illinois Agricultural Experiment Station. Bulletin 87, 1903.

Inasmuch as this material had been extracted with a large quantity of water it was very thoroughly freed from water-soluble proteins, and since the embryos were practically all removed intact very little of the protein in this "corn gluten" could have been derived from this part of the seed. Nearly all of the protein was consequently derived from the endosperm, and consisted chiefly of zein and "maize glutelin." By extracting with 10 per cent potassium chloride solution we removed only 4.8 per cent of the nitrogen, or 0.35 per cent of the "corn gluten," equal to 2.2 per cent of protein containing 16 per cent of nitrogen.

In this "corn gluten" the ratio of zein to "maize glutelin" is 100:44 which is very much higher than was found in our analysis of the endosperm meal of the high nitrogen corn, namely 100:74. Whether the losses of maize glutelin incident to the processes employed in making this preparation of corn gluten were greater than those of the zein, or the proportion of zein to maize glutelin in the high nitrogen corn was less than in the sample of low nitrogen yellow corn which we used for our earlier analysis, cannot be determined from any data at present available. It is to be noted that in our earlier analysis 52 per cent of the total nitrogen was soluble in alcohol, whereas in the high nitrogen white corn only 41 per cent was soluble therein. If such a difference actually exists it ought to have a pronounced influence on the food value of these two types of corn, especially when fed to growing animals, since, as will be shown in this paper, growth proceeds at a rate determined by the degree in which the amino-acid deficiencies of zein are quantitatively supplemented by the addition of other proteins. These considerations show how important it is to have abundant and exact data concerning the relative proportions of the different types of nitrogen in the seeds of the numerous varieties of corn.

In a previous paper[5] we have pointed out that the problems of the dietary in efficiency of zein hinge upon the peculiarities of its amino-acid make-up. When zein serves as the sole source of nitrogen in the ration, nutritive failure inevitably results sooner or later. This is not due to the failure to digest the protein or absorb its cleavage products. Zein is commonly regarded as rather difficult to digest; but when properly hydrated it is fairly well

Osborne and Mendel: this *Journal*, xvii, p. 325, 1914.

utilized. Thus for albino rats, which served as subjects in all our experiments, the "coefficient of digestibility," or utilization, calculated in the conventional way, ranged from 71 to 84 per cent, in contrast to 92 per cent for lactalbumin and 90 per cent for casein or gliadin. Obviously the unfavorable nutritive result cannot be charged to a lack of absorption of the digestion derivatives of zein.

The compilation of the most recent data in reference to the quantities of different amino-acids obtainable from zein by hydrolysis is given in our earlier paper.[6] This brings out the complete lack of glycocoll, lysine, and tryptophane among them, as well as the relatively small yield of arginine and histidine. The character of the nutritive failures with our zein foods, as exemplified in declining body-weight, is exhibited in Chart IV appended to the publication just referred to.

With respect to the specific significance of the individual missing amino-acids little need be said about *glycocoll.* The consensus of opinion and the weight of experimental evidence, particularly as exemplified in hippuric acid production in the body, indicate that it can be synthesized anew by mammals. Its absence from the diet can therefore be made good within the organism itself. *Tryptophane* is indispensable, as we have already pointed out.[7] No better illustration of its unique rôle in maintenance could be afforded than is given by rat 1892 in Chart V in the paper just referred to in which the inevitable failure and decline in body-weight on zein food was shown to be checked for 111 days when tryptophane equivalent to 3 per cent of the protein is added to it.[8] In this experiment, which is still in progress, the body-weight of the animal has been maintained unchanged for 154 days. The entire absence of growth on foods containing zein + tryptophane in this and comparable experiments already published, in which young rats are nevertheless adequately maintained, indicates clearly the pronounced difference between the amino-acid requirement in growth and in maintenance. Only when all the necessary amino-acids are furnished can new construction of tissue proceed. In the

[6] This *Journal,* xvii, p. 325, 1914.

[7] *Ibid.*

[8] Further data and discussion bearing on this are given in this *Journal,* xvii, p. 325 *et seq,* 1914.

case of zein, therefore, the missing lysine must be supplied before growth takes place. *Lysine* will not replace tryptophane in making maintenance possible, as is shown by the failure of rat 1900 in Chart VI of the earlier paper.[9] The function of these two amino-acids is elucidated when both are fed along with zein-food and growth results. We have appended a few new feeding trials in this direction (although the subject was dealt with in the earlier report) because they show such surprising success and emphasize so strongly the dominant importance and individual part played by certain amino-acids in nutrition—and further because the newer experiments contain the innovation of having additions of histidine and arginine in which zein is relatively, though not absolutely, deficient. (See Chart I.)

The decline in body-weight experienced by animals on a diet containing zein alone, along with non-nitrogenous foodstuffs, can be stopped not only by suitable additions of lacking amino-acids, but also by supplementing the ration with other proteins. This is, of course, precisely what happens when the maize kernel itself is fed. The zein in the seed is accompanied by an approximately equal quantity of other proteins: maize glutelin, albumins, globulins, "proteose," etc. Regarding the chemical structure of the latter nothing is known at present, except that maize glutelin yields all of the familiar amino-acid derivatives of protein. The induction of growth on a diet containing maize glutelin as the sole protein is shown by rats 547 and 567, Chart II.

It is possible to secure satisfactory growth of young rats on diets in which zein forms a very considerable portion of the ration provided that other suitable proteins are supplied in addition, as shown in a recent paper[10] and further in the appended Chart III of experiments in which the supplementary protein consisted of casein. The relative proportions of these proteins requisite to convert the inadequate zein ration into a growth-promoting food vary quite widely and are dependent on their comparative content of the amino-acids in which zein is entirely or partially deficient.

On a diet having a protein concentration of 18 per cent of the solids of the ration, for example, growth may be slower than normal

[9] Osborne and Mendel; this *Journal*, xvii, p. 325, 1914.

[10] *Ibid.*

when the relative proportion of the supplementary protein added to the zein is smaller than that indicated in the satisfactory growth just referred to. The retardation becomes more conspicuous in each case with the proportionate decrease in the content of adequate protein. This is shown in Chart VIII already published.[11] A protein deficient in lysine, as is gliadin, fails to promote growth in any degree. (See Chart I[12]) If, as in this case, it contains sufficient tryptophane, it may suffice for maintenance alone. The rate of growth on otherwise suitable diets of zein containing additions of other proteins is therefore primarily dependent on the quantitative amino-acid make-up of the supplementary protein. The significance of this has already been emphasized in an earlier paper.[13] The relative deficiency of the zein + casein combination in tryptophane there described in Chart VIII is exemplified by the accelerated growth which resulted on the addition of tryptophane. In the zein + edestin combination the shortage involves the lysine factor and can be made good by supplying this compound. (See Chart III of the earlier paper.)

In Chart IV are shown additional experiments in which zein has been supplemented by maize glutelin, vetch legumin, and phaseolin respectively. The body-weight of the animals was maintained in every case, and slow growth was secured in some instances, *e.g.* with a food containing equal parts of zein and maize glutelin. Foods in which the protein was either zein or phaseolin alone invariably proved inadequate for maintenance. When equal parts of both these proteins were present in the diet the animal was maintained, but growth was not secured. (See Chart IV.) Inasmuch as phaseolin is not entirely deficient in any of the amino-acids known to be yielded by proteins its ability to supplement zein so as to produce maintenance can be understood. No growth, however, has been secured. The failure of phaseolin alone to suffice for either maintenance or growth remains to be explained.

It is not necessary to use pure zein in order to exemplify the nutritive shortcomings of the chief protein of maize. The "corn gluten," to which reference has already been made on page 5 contains sufficient maize glutelin to permit of maintenance on

[11] This *Journal*, xvii, p. 325, 1914.
[12] *Ibid.*
[13] *Ibid.*

this ration. More than very slow growth is impossible, however. (See Chart V.) Additions of other proteins to "corn gluten" have been made. The outcome is shown in Chart VI in which growth has resulted in accord with what one might expect from the amino-acid make-up of the proteins added. Lactalbumin, rich in both tryptophane and lysine, is a most efficient adjuvant; casein or edestin must be added in far larger proportion to accomplish results approaching those of the milk albumin.

The foregoing experiences bring into new light certain problems related to the economy of foods and commercial fodders. Corn forms the cheapest basis for the feeding of farm animals in food production. Inasmuch as the rate of growth is limited by hereditary, rather than nutritive, conditions, it is futile to furnish more energy, and particularly more protein, than is essential for normal development. An inadequate. but cheap protein, can be supplemented advantageously by one which supplies the needed factors, *i.e.*, amino-acids. The relative economy of these additions of supplementary proteins to an inefficient but inexpensive ration depends not only on their quantity but likewise on their amino-acid make-up. A very small addition of a protein like lactalbumin may be far more advantageous, when the cost per unit of gain is considered, than larger amounts of cheaper proteins which supplement the amino-acid deficiency of the standard diet less perfectly. It is perhaps not too utopian to expect that the day may come when amino-acid concentrates may serve to render perfect the mixtures of proteins in a fodder like maize or its commercial by-products.

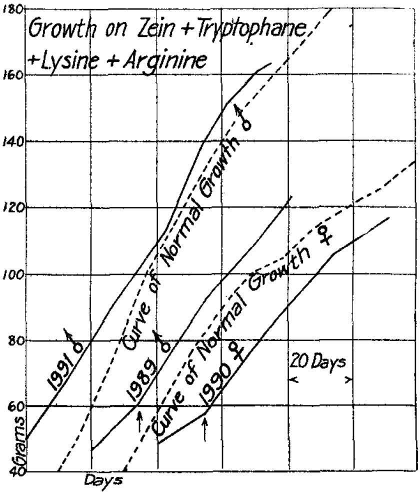

Chart I. Growth on Foods containing Zein + Tryptophane + Lysine + Arginine. For rats 1989 and 1990 at the periods indicated by the arrows *histidine* was added to the diet.

The food contained:

	Without histidine *grams*	With histidine *grams*
Zein	16.65	16.47
Tryptophane	0.54	0.54
Lysine	0.54	0.54
Arginine	0.27	0.27
Histidine	0.00	0.18
Protein-free milk	28.00	28.00
Starch	26.34	26.15
Butter-fat	18.00	18.00
Lard	9.00	9.00
Water	15.00	15.00

At present we attach no importance to the slight increase in the rate of growth shown by rats 1989 and 1990 after the addition of histidine to the diet, inasmuch as rat 1991 made quite as rapid growth without this amino-acid addition.

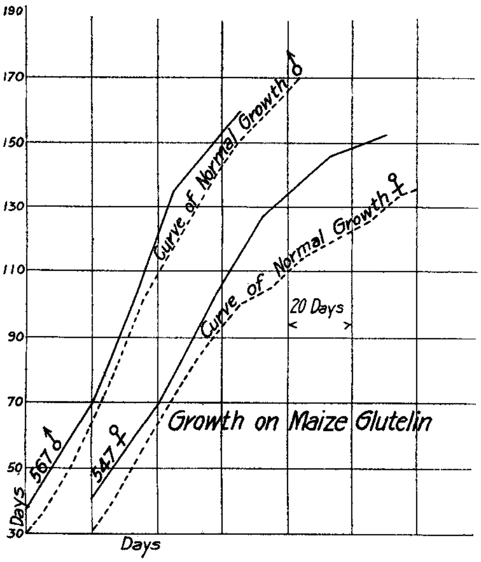

CHART II. GROWTH ON FOOD CONTAINING MAIZE GLUTELIN AS ITS SOLE PROTEIN.

The food contained:

	Per cent
Maize glutelin	18
Protein-free milk	28
Starch	28
Lard	26

CHART III. (P. 13). EFFECT ON GROWTH OF REPLACING ZEIN WITH DIFFERENT PROPORTIONS OF CASEIN.

The foods contained:

	1616, 1596 *grams*	1851, 1836, 1835, 1533, 1566 *grams*
Zein	4.5	9
Casein	13.5	9
Protein-free milk	28.0	28
Starch	28.5	28
Butter-fat / Lard	25.5	26
Water	3.8	7.5.

Rats 1533 and 1566 received no butter-fat or other growth-promoting fat in the diet. For rats 1616 and 1596 butter-fat was added at the points indicated by the arrows, when the failure of growth already described in earlier papers (this *Journal*, xvi, p. 423, 1913) manifested itself.

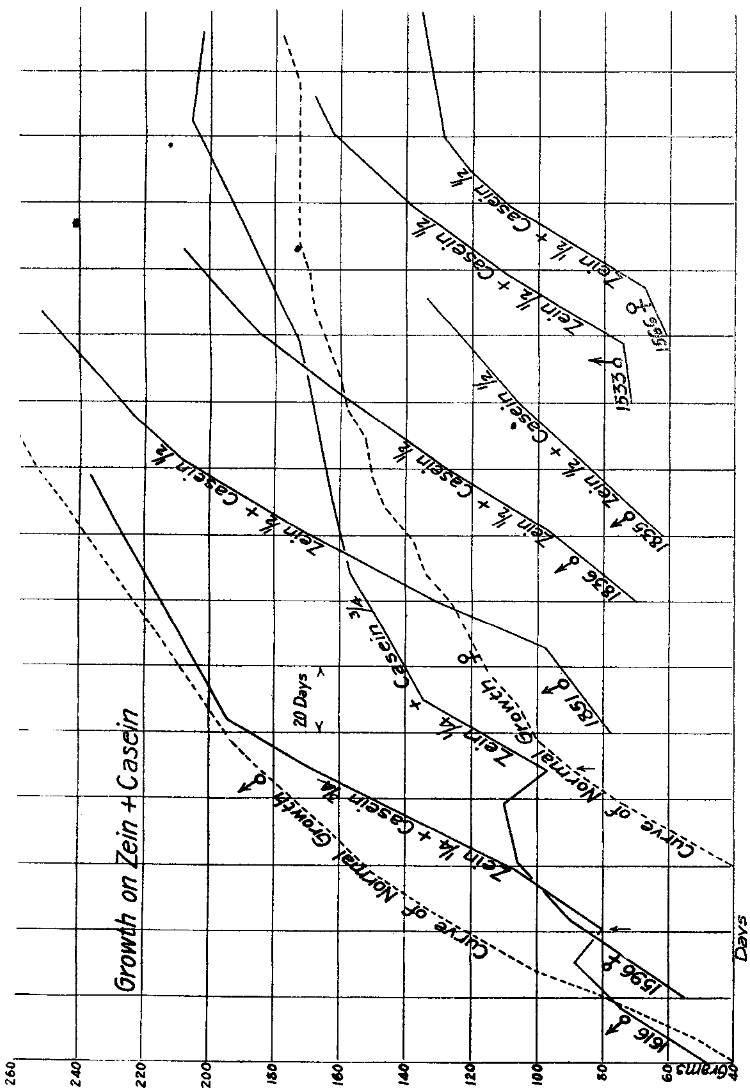

Chart III. Legend on page 12.

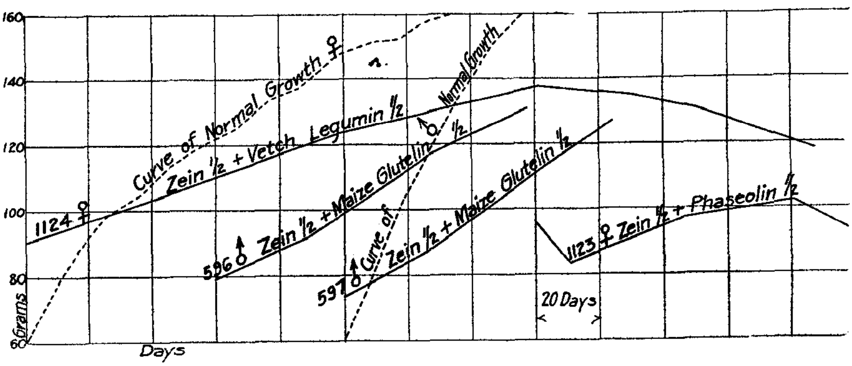

CHART IV. COMPARATIVE EFFECT OF MAIZE GLUTELIN, PHASEOLIN, AND VETCH LEGUMIN IN PREVENTING THE NUTRITIVE DECLINE CHARACTERISTIC OF ZEIN FOOD.

The foods consisted of:

	1124	596 597	1123
	grams	*grams*	*grams*
Zein	9.0	9	9.0
Maize glutelin	0.0	9	0.0
Phaseolin	0.0	0	9.0
Vetch legumin	9.0	0	0.0
Protein-free milk	28.0	28	28.0
Starch	27.5	24	27.5
Lard	26.5	30	26.5
Water	7.5	10	7.5

Although the proportion of the supplementary proteins used is alike in these experiments, maize glutelin is far more efficient in promoting growth than are the leguminous proteins here tested. It is interesting to note the maintenance of rat 1123 on a diet containing a mixture of proteins, either of which alone is inadequate for maintenance.

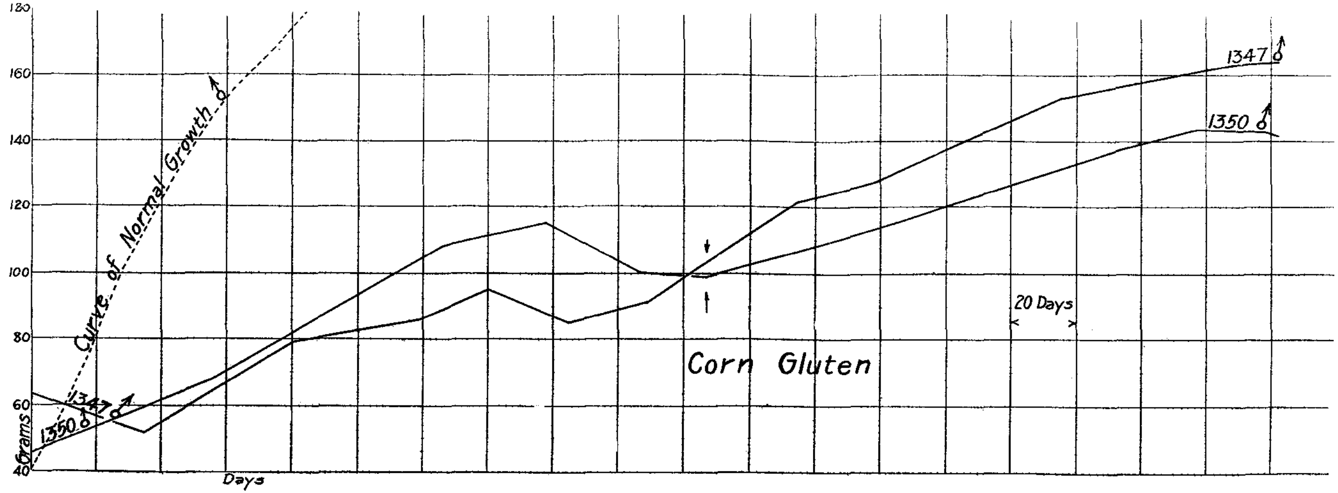

CHART V. MAINTENANCE AND SLOW GROWTH ON FOOD CONTAINING "CORN GLUTEN."

The food consisted of:

	per cent
"Corn gluten" (see page 5)	38
Protein-free milk	28
Starch	2
Lard	32

Butter-fat, which has been found to promote growth was added to replace 18 per cent of lard, at the periods indicated by the arrows.

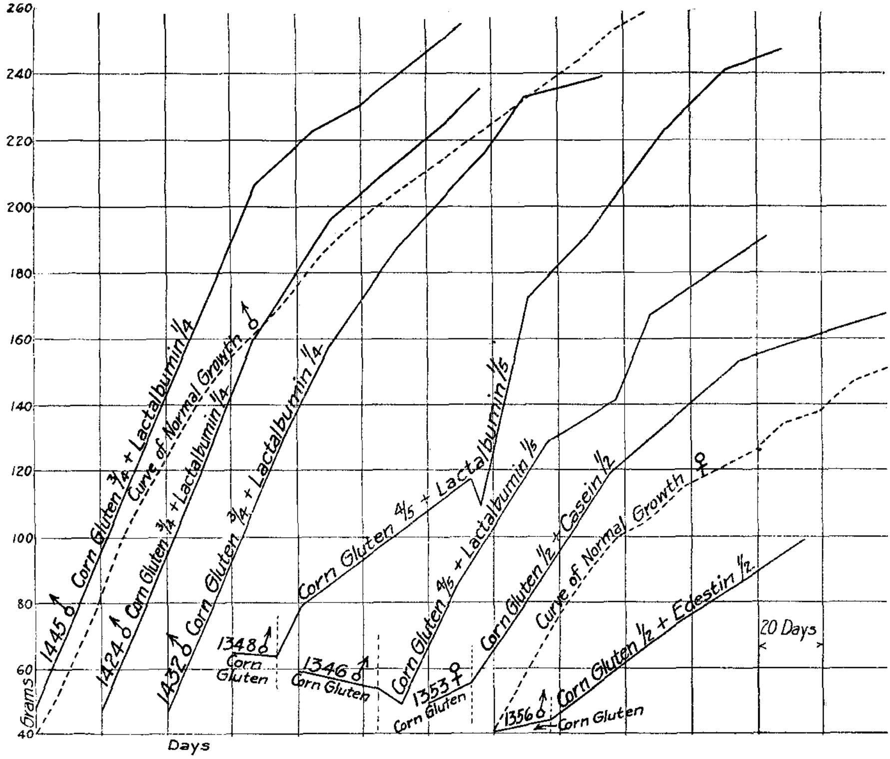

CHART VI. EFFECT OF SUPPLEMENTING "CORN GLUTEN" WITH PROTEINS ADEQUATE FOR GROWTH.

The food consisted of:

	1445 1424 1432	1348 1346	1353	1356
	per cent	*per cent*	*per cent*	*per cent*
"Corn gluten" (See page 5)	28.5	30.4	19.0	19
Lactalbumin	4.5	3.6	0.0	0
Casein	0.0	0.0	9.0	0
Edestin	0.0	0.0	0.0	9
Protein-free milk	28.0	28.0	28.0	28
Starch	8.5	7.2	17.5	16
Lard	30.5	30.8	26.5	28

The effect of corn gluten alone (see composition of food for Chart V) is shown in the earlier parts of the curves for rats 1348, 1346, 1353, and 1356. It will be noted that, for reasons explained in the text, the smaller supplementary portions of lactalbumin are even more effective than larger admixtures of casein and edestin in promoting growth. The rapid recovery of rat 1348 from the decline noted at the end of 77 days is due to the substitution of natural protein-free milk for the artificial protein-free milk IV described in this *Journal*, xv, p. 317, 1913.