NOTE TO USERS

INHERITANCE OF PROTEIN, ZEIN, TRYPTOPHAN, VALINE, LEUCINE, AND ISOLEUCINE IN TWO MAIZE HYBRIDS

by

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INTRODUCTION

Practical experience and scientific research (35, 38, 39, 40, 58) have demonstrated that certain plant proteins, such as those of the corn kernel, fail to promote animal growth effectively unless they are supplemented by added nutrients. These deficiencies may be of more than one sort; but among them the inadequate or qualitatively inappropriate character of the protein is a clearly demonstrated feature. In order to understand the nature of this inadequacy, it should be noted that proteins are very complex compounds which have as their basic building units amino acid molecules. Some 22 amino acids are recognized as playing a part in protein structure, and of these 10 are established as indispensable to growing rats, i.e., they cannot be synthesized by the rat but are necessary for life and growth. If the protein source in a diet does not contain sufficient amounts of the indispensable amino acids to maintain normal growth, those lacking must be supplied to supplement the original protein source.

Corn is an example of a source of nutritionally unbalanced protein, which by the addition of certain amino acids, notably tryptophan and lysine, promotes normal growth in animals. The livestock feeder who uses only corn in his livestock ration is faced with the problem of how to balance the protein in the ration. A nutritionally balanced ration may be obtained in one of several ways:

1. The feeder can supplement corn in the ration with a protein or amino acid concentrate.

2. The feeder can supplement corn in the ration with home-grown proteins from soybeans, flax, etc.

3. Since it is a well established fact that chemical composition of corn is under genetic control, the corn breeder can attempt to modify the protein quality in corn to overcome its deficiencies. Such a program was inaugurated at the Iowa Agricultural Experiment Station in 1945.

With the inception of this program certain questions arose about the best breeding procedures to follow. Individual plants were studied in the P_1, P_2, F_1, F_2, Bc_1, and Bc_2 of the crosses high protein by low protein, and Hy x Il98, with the hope of obtaining partial answers to the relevant questions. The phases of the program under study were as follows:

1. The extent of relationship between total protein, zein, tryptophan, valine, leucine, and isoleucine in corn.

2. The relation between protein percentage and yield of grain per plant.

3. The relation of protein and non-zein protein percentages to tryptophan content of corn.

4. The effect of xenia upon the protein of the corn kernel.

5. The effect of seasonal differences upon protein and tryptophan percentage.

6. The range of tryptophan percentages in Corn Belt inbred lines and a survey of available inbreds to determine their protein-zein balance.

7. The nature of inheritance of total protein, zein, tryptophan, valine, leucine, and isoleucine percentages.

Because of the range of subject matter under investigation it is appropriate to divide this manuscript into two parts; the first present-

ing data pertinent to items one through six above, and the second presenting data of special significance to the planning of future corn improvement programs in respect to inheritance of protein quality. This scheme gathers the more or less practical aspects of the study into the first and the theoretical aspects into the second category.

PART I. VARIATION OF PROTEIN AND CERTAIN OF ITS COMPONENTS AMONG INBREDS AND SEGREGATING POPULATIONS

Review of Pertinent Literature

The first studies upon the protein of corn were made by Gorham (32) in 1822. He reported the presence of an alcohol soluble protein which made up 3.3 percent of the corn kernel and 40 percent of the corn protein, to which he gave the name zein. A few years later Bizio (3) reported 5.7 percent zein in a sample of corn.

Using Fischer's esterification procedure, Osborne and Clapp (47) analyzed zein for 13 amino acids and discovered high percentages of leucine and glutamic acid, but no tryptophan or lysine. This probably was the first hint of the nature of the nutritional deficiency of corn protein; however, this fact was not pointed out until later (48).

After the discovery that zein was deficient in lysine and tryptophan, feeding trials were needed to prove that this deficiency was the cause of poor protein quality in corn. Willcock and Hopkins (58)

reported that mice died in a short time when fed zein or zein plus tyrosine rations, but zein plus tryptophan rations prolonged life. This finding was further substantiated in feeding experiments by Osborne and Mendel (49), Hogan (25), and Marais and Smuts (38), all of whom found that when zein or whole corn was supplemented by lysine and tryptophan, normal growth occurred.

In order to compare the quality of proteins from different sources, some measure of their relative nutritive value was needed. For this purpose Mitchell (43) devised the following formula:

$$\text{Biological value} = \frac{\text{Total N intake} - (\text{Urinal N from food} + \text{fecal N from food})}{\text{Total N intake} - \text{fecal N from food}} .$$

By using this formula to analyse rat feeding trials, the following biological values were obtained:

Protein source	% protein in ration	Biological value	Reference
Corn	5	72	Mitchell (44)
Corn	10	60	Mitchell (44)
Corn	-	67	Marais & Smuts (38)
Corn + tryptophan	-	70	Marais & Smuts (38)
Corn + lysine	-	66	Marais & Smuts (38)
Corn + lysine + tryptophan	-	81	Marais & Smuts (38)
Corn	-	54	Mitchell & Kick (45)
Corn	-	67	Boas Fixen & Jackson (6)

In 1914 Osborne and Mendel (48) divided the proteins of corn germ and endosperm according to their solubility with the following results:

Protein	Kernel part	
	Endosperm % of protein	Embryo % of protein
Globulins	7.8	77.2
Zein	50.0	2.0
Glutelin	38.2	0.6
Insoluble	4.0	20.2

These results showed germ protein to contain only a small amount of zein, so they concluded it to be balanced nutritionally. This was proven true by Hogan's feeding experiments (25). Block and Bolling (4) gave nutritive values of 1.95 and 1.90 for corn germ protein and milk protein, respectively. From the studies reported, it is apparent that germ protein could not supplement the endosperm protein which contains so much zein.

Marais and Smuts (40) found that soybeans plus corn in a 9.75 percent protein ration gave a biological value of 75, compared to 67 and 55 for corn and soybeans, respectively. However, McCollum, et al. (35) found that neither flaxseed nor millet seed served as a good source of protein supplement for corn when fed in a nine percent protein ration.

Up to this point, corn protein had been shown to be of poor nutritional quality, but no consideration had been given to the relative quality of corn protein from different sources. In this connection, Showalter and Carr (52) showed that corn protein quality decreases as the percentage of protein in corn increases when they discovered low protein corn to contain 30 percent of its protein as zein, while

high protein corn contained 50 percent of its protein as zein. In more recent studies, Hansen, Brimhall and Sprague (22) found a straight line relationship between zein and protein in corn which ranged from 6.0 to 19.0 percent protein. The regression value was 0.478. This means that as corn protein percentage increases, a large share of the increase is actually in the form of zein.

Of more academic interest are the relative amounts of zein present in corn at different stages of maturity. Zeleny (60) studied the distribution of nitrogen in corn at four stages of maturity:

A - kernels ½ normal size
B - kernels full grown, but still milky
C - kernels starting to dent
D - mature corn

The following results were obtained by extraction with different solvents:

Expressed as % of total N

Sample	H_2O soluble non-protein	Protease and peptone	Globulin	Zein	Glutelin	Not soluble
A	41.1	1.5	7.7	2.8	13.0	34.0
B	31.2	2.2	6.6	13.5	18.2	28.4
C	14.9	1.3	8.4	30.2	17.0	28.3
D	4.6	1.7	7.8	42.0	16.9	27.0

These data show that water soluble non-protein nitrogen decreases rapidly while zein increases rapidly with maturity. Similar results were shown by Spitzer, Carr, and Epple (54), who, using improved chemical methods of analysis, found that soft corn contained 22 percent

medium soft corn 29 percent, and mature corn 41 percent zein.

The following table is included from Block and Bolling (4) to show the amino acid composition of corn and corn protein fractions:

Table 1. Approximate percentage of amino acids in corn proteins

Amino Acid	Whole Corn	Germ	Glutelin	Zein	Zein residue	Albumins
Arginine*	4.0	6.8	3.1	1.6	2.9	5.4
Alanine	-	-	-	9.9	-	-
Aspartic acid	-	-	-	3.4	-	-
Cystine	1.1	1.2	1.2	0.8	1.8	0.5
Glutamic acid	-	-	24.5	35.6	-	-
Glycine	-	-	4.3	0.0	9.6	-
Hydroxyproline	-	-	-	1.0	-	-
Isoleucine*	3.6	3.7	4.9	4.3	2.0	1.3
Leucine*	21.5	16.3	24.7	23.7	11.0	11.3
Lysine*	2.5	5.8	1.1	0.0	1.6	1.0
Methionine*	-	2.3	5.5	2.4	4.8	-
Phenylalanine*	4.5	5.6	6.6	6.4	4.5	1.7
Proline	-	-	-	11.5	-	-
Histidine*	2.4	2.7	1.7	0.9	1.6	6.7
Threonine*	3.6	4.4	4.0	2.4	4.0	3.9
Tyrosine	6.1	4.9	6.2	5.0	6.2	3.8
Tryptophan*	0.6	1.3	0.6	0.1**	1.1	0.7
Valine*	4.6	5.8	4.6	2.4	5.5	2.5

*Indispensable amino acid
**Usually reported as 0.0

This table was compiled from many different sources, and therefore values should be taken as approximations. This point is illustrated by the case of tryptophan in zein. The table shows zein to contain 0.1 percent tryptophan, and May and Rose (42) show 1.0 percent, while

Jones and Csonka (28), Matsuyama and Mori (41), and Osborne and Mendel (49) found none. The reasons for such disparities are: (1) different analytical procedures and different sources of material give different results; (2) protein fractions are many times impure. However, it is generally reported that zein contains no tryptophan and whole corn contains less than 0.1 percent tryptophan.

Bollenback (7), using microbiological assay methods, analyzed a sample of each of Illinois high and low protein corns and concluded that high protein corn contained a higher percentage of tryptophan, valine, leucine, isoleucine, and lysine than did low protein corn.

Materials and Methods

Materials

The corn samples[1] analyzed in the present study represent material from two sources. Group I includes:

1. P_1, Illinois high protein corn
2. P_2, Illinois low protein corn
3. F_1, Illinois high protein x Illinois low protein
4. F_2, Illinois high protein x Illinois low protein
5. Bc_1, backcross to Illinois high protein
6. Bc_2, backcross to Illinois low protein

[1] A sample refers to the grain produced on an individual plant, i.e., an F_1 sample is a selfed ear grown on an F_1 plant. In reality, the protein-producing ability of the genotype of the F_1 plant is being measured.

This material was obtained from the Illinois Experiment station in 1940 and inbred two generations before the cross was made. These two parents were selected because they represented extreme differences of protein percentages. Group I was grown in the field in 1946.

Group II includes:

1. P_1, I198
2. P_2, Hy
3. F_1, I198 x Hy
4. F_2, I198 x Hy
5. Be_1, backcross to I198
6. Be_2, backcross to Hy

Hy and I198 are Corn Belt inbreds. I198 was selected because it represented the highest protein percentage of lines then analyzed. Group II was grown in the field in 1945. Throughout the remainder of this paper the letter designations "P_1", "P_2", etc., as given above, will be used.

Twenty-five kernels of each of the P_1, P_2, F_1, and 150 kernels of each of the F_2, Be_1, and Be_2 were planted in adjacent 25-foot rows with the kernels spaced one foot apart. This required six rows for an F_2, or backcross, and one row for a parent, or F_1. The plants were selfed to eliminate any effects of foreign pollen, either upon the protein percentage and composition of the kernels in the immediate generation, or upon the selected material in the next generation. After losses due to cultivation, barren plants, and the failure of

pollen and silks to nick, about 15-20 samples were obtained of each of the P_1, P_2, and F_1, and about 80-100 samples of each of the F_2, Bc_1, and Bc_2.

Samples were harvested when mature and dried to 10-12 percent moisture. Each ear was given a selection number, shelled individually, and the weight of grain per ear recorded in grams.

Data obtained in Group I material were protein, zein, tryptophan, valine, leucine, and isoleucine percentages. Data obtained on Group II material were protein, zein, and tryptophan percentages. Also in Group II, from the array of F_2 protein percentages, every third sample was selected to analyze for valine, leucine, and isoleucine. Lysine determinations were attempted, but the analytical method and the material to be analyzed proved to be incompatible.

In order to study the effect of foreign pollen upon protein percentage and composition, paired comparisons were made between selfed and crossed seeds grown upon the same ear. This material was obtained by pollinating Illinois high protein ears (white dent corn) with a mixture of pollen from the ear-bearing plant and pollen from Krug variety (yellow dent corn). Separation of crossed from selfed seeds was effected by separating the white from yellow kernels on the ear.

For the study of yearly effect upon protein and tryptophan percentages, seeds of inbreds were taken from breeding stocks. These inbreds were not grown in a replicated experimental field design and were not necessarily grown in adjacent rows.

The inbreds used in making up the survey of breeding material available for protein quality improvement represent those most widely used in the Corn Belt. All of those included were grown in the summer of 1946 in the breeding nursery.

Methods of chemical analysis

Each air dried sample was thoroughly mixed and a two-ounce bottle filled from each sample by dipping into the mixed grain. These two-ounce sub-samples were ground to pass a 20-mesh sieve on a Christy-Norris laboratory mill.

Protein analysis. Total protein was determined by the Kjeldahl method as described by Willard and Furman (57). Protein percent was obtained by multiplying nitrogen by 6.25.

Zein analysis. Alcohol soluble protein, or zein, was determined by the method of Hansen, et al. (22) with slight modifications. To two grams of corn in a 125 ml. glass stoppered flask was added 50 ml. of 70 percent ethyl alcohol (sp. gr. = .865). The flasks and contents were shaken on a mechanical shaker for 30 minutes, allowed to stand over night, and again shaken for 30 minutes. The contents then were filtered and 33 ml. aliquots of the filtrate taken for nitrogen analysis by the Kjeldahl method. One hundred ml. of water were added to the flasks before digestion to prevent foaming.

Tryptophan analysis. The method of Sullivan, et al. (56) modified by Brimhall (unpublished) was used to determine tryptophan content of the corn samples. The modifications employed were: (1) The corn

samples were pealed before grinding because presence of bran was found to interfere with the intensity of blue color obtained in this procedure. (2) The p-dimethylaminobenzaldehyde was dissolved directly in the hydrochloric acid instead of in 10 percent sulfuric acid. (3) Instead of casein, corn was used as a standard. Corn samples known to cover a wide range of tryptophan percentages were selected and transmission percentages determined by Sullivan's method. These transmission values were plotted against amounts of tryptophan in the same corn samples as determined by the microbiological assay procedure of Greene and Black (21). The regression line plotted from these points is shown in Figure 2.

The conversion of the transmission percentages to percent of tryptophan was made by the use of values in Figure 2.

There is some question among certain workers in the field of tryptophan assay investigation as to the accuracy of the tryptophan values obtained by this method. This objection may not be too serious in selection experiments since selection for tryptophan is done on a relative basis. However, throughout the remainder of this thesis, tryptophan percentage should be interpreted to mean apparent tryptophan percentage.

Valine, leucine, and isoleucine analyses. The microbiological assay method of Kuiken, et al. (30), employing Lactobacillus arabinosus 17-5, with modifications by Bollenback (7), was used to determine leucine, isoleucine, and valine.

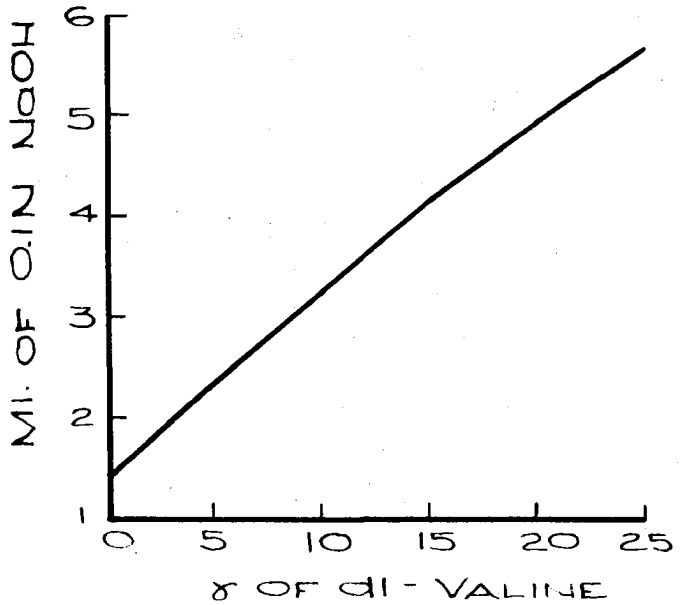

Figure 1. Valine standard curve

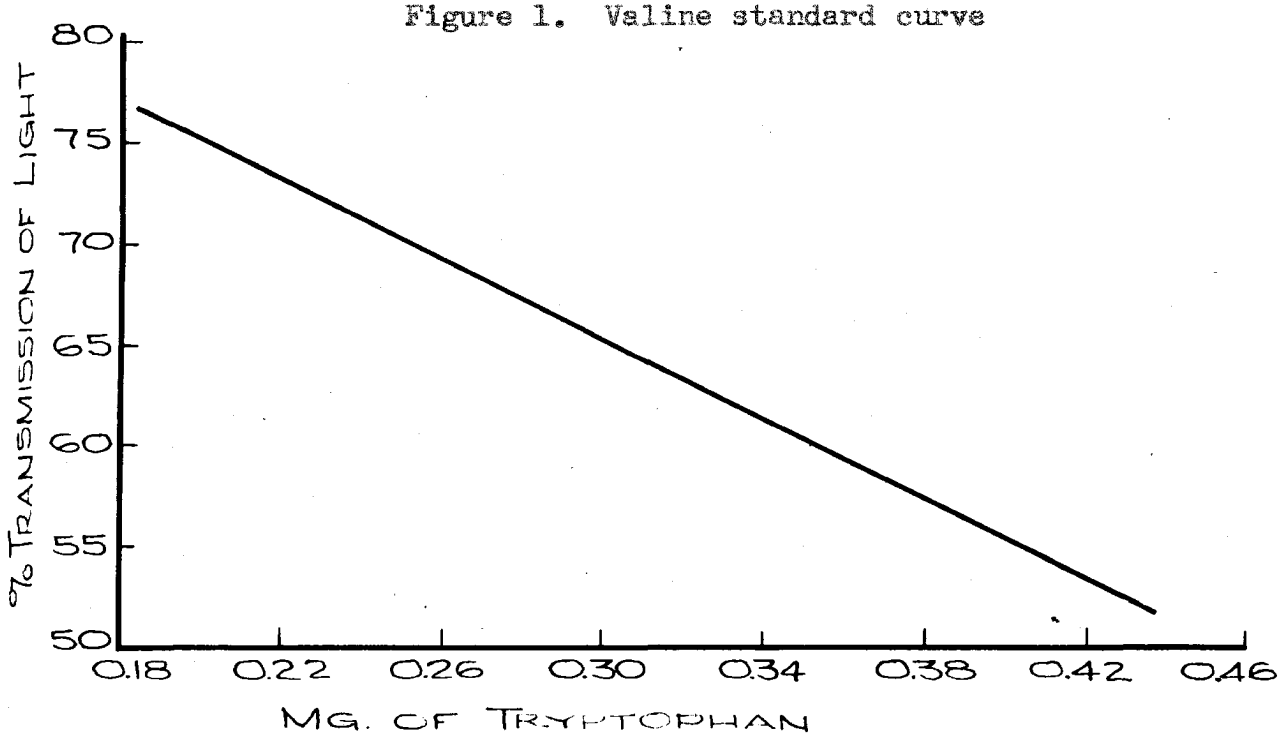

Figure 2. Tryptophan standard curve

Microbiological assay depends upon the well known fact that an organism, plant or animal, grows and respires in proportion to the indispensable nutrient most deficient in its medium. Use of an organism for assaying purposes involves two problems: (1) determining nutrients indispensable to the assaying organism, and (2) obtaining a method of measuring growth and respiration.

The modified nutrient requirements of Lactobacillus arabinosus 17-5 are given in Table 2. Other modifications of this method were: (1) The medium of McMahan and Snell (36), as given in Table 3, was used for maintenance of stock cultures. (2) The samples were hydrolyzed with 2N hydrochloric acid for four hours in an autoclave at 15 pounds pressure and sodium hydroxide was used to neutralize the hydrolyzates. (3) Phenolphthalein was used as an indicator when titrating. (4) The medium components were mixed dry and then dissolved. For a discussion of these modifications, see Bollenback (7).

A typical standard curve is shown in Figure 1. In the present study, the percentage values for valine, leucine, and isoleucine in a sample are each the average of four tubes.

Table 2. Suggested media for determining valine, leucine, and isoleucine with Lactobacillus arabinosus 17-5

Component	Concentration per liter of basal medium
Glucose, gm.	40.0
Sodium acetate, gm.	14.5
Adenine sulfate, mg.	10.0
Uracil, mg.	10.0
Guanine hydrochloride, mg.	10.0
Thiamine chloride, γ	200.0
Pyridoxine hydrochloride, γ	200.0
Calcium pantothenate, γ	200.0
Biotin, γ	0.8
Riboflavin, γ	400.0
Niacin, γ	800.0
p-Aminobenzoic acid, γ	1.0
K_2HPO_4, gm.	1.0
KH_2PO_4, gm.	1.0
$MgSO_4 \cdot 7H_2O$, mg.	400.0
NaCl, mg.	20.0
$FeSO_4 \cdot 7H_2O$, mg.	20.0
$MnSO_4 \cdot 4H_2O$, mg.	20.0
l-Arginine hydrochloride, mg.	400.0
dl-Alanine, mg.	400.0
dl-Aspartic acid, mg.	800.0
dl-Glutamic acid, mg.	800.0
l-Histidine monohydrochloride, mg.	400.0
l-Lysine hydrochloride, mg.	400.0
dl-Phenylalanine, mg.	400.0
l-Proline, mg.	400.0
dl-Serine, mg.	400.0
l-Tryptophan, mg.	400.0
dl-Methionine, mg.	400.0
dl-Tyrosine, mg.	400.0
dl-Threonine, mg.	400.0
l-Cystine, mg.	400.0
dl-Valine, mg.	400.0
dl-Leucine, mg.	400.0
dl-Isoleucine, mg.	400.0
Adjust pH to 6.8 and dilute with water to one liter	

Table 3. Suggested medium for culturing *Lactobacillus arabinosus* 17-5

Component	Concentration per 100 cc. of medium
Bacto-peptone, gm.	0.8
Yeast extract, gm.	0.1
Sodium acetate, gm.	0.1
Glucose, gm.	1.0
K_2HPO_4, mg.	1.0
KH_2PO_4, mg.	1.0
$MgSO_4 \cdot 7H_2O$, mg.	0.2
NaCL, mg.	0.01
$FeSO_4 \cdot 7H_2O$, mg.	0.01
$MnSO_4 \cdot 4H_2O$, mg.	0.01

Experimental Results

The means and ranges of protein, zein, apparent tryptophan, valine, leucine, and isoleucine percentages for Group I material are given in Table 4, and the means and ranges of protein, zein, and apparent tryptophan percentages for Group II material are given in Table 5. The results are expressed as percent of corn on a dry weight basis. Since corn, instead of casein, was used for a standard curve, direct comparisons of these tryptophan values should not be made with those of other studies.

Interrelations of protein, zein, tryptophan, valine, leucine and isoleucine

The interrelationships of protein, zein, tryptophan, valine, leucine and isoleucine percentages in corn were studied from two aspects: (1) the degree of covariation of these variables, and (2) the variation of protein components relative to protein variation.

To measure the degree of covariation correlation coefficients were employed. All possible correlation coefficients among protein, zein, tryptophan, valine, leucine, and isoleucine percentages were calculated. These are given in Tables 6, 7, and 8 for the F_2, Bc_1, and Bc_2 generations, respectively, of Group I, and in Tables 9, 10 and 11 for the F_2, Bc_1, and Bc_2 generations, respectively, of Group II.

Correlations involving protein with zein and each of the amino

Table.4. Means and ranges of protein, zein, tryptophan, valine leucine, and isoleucine percentages for each population in Group I

		Protein	Zein	Tryptophan	Valine	Leucine	Isoleucine
P_1	mean	17.2	7.4	0.122	0.83	2.99	0.98
	range	14.2-19.2	5.6-9.1	0.108-0.146	0.71-1.04	2.01-3.86	0.75-1.25
P_2	mean	7.2	2.1	0.084	0.34	0.79	0.39
	range	5.5-10.5	1.0-4.2	0.057-0.156	0.20-0.49	0.43-1.24	0.30-0.62
F_1	mean	6.6	1.7	0.074	0.34	0.96	0.32
	range	4.4-10.0	0.8-3.6	0.057-0.091	0.21-0.52	0.65-1.76	0.21-0.49
F_2	mean	9.6	3.3	0.087	0.45	1.42	0.48
	range	5.9-16.0	1.0-6.7	0.060-0.122	0.27-0.69	0.60-2.66	0.27-0.84
Bc_1	mean	11.8	4.3	0.098	0.58	1.72	0.66
	range	8.2-18.4	2.1-8.7	0.073-0.118	0.40-0.82	0.78-3.58	0.48-1.04
Bc_2	mean	6.8	1.9	0.072	0.34	0.88	0.42
	range	4.9-10.2	0.9-3.5	0.057-0.087	0.22-0.54	0.52-1.47	0.27-0.65

Table 5. Means and ranges of protein, zein, and tryptophan percentages for each population in Group II

	Protein	Zein	Tryptophan
P_1 mean	12.8	4.9	0.114
range	11.7-13.5	4.1-5.6	0.105-0.122
P_2 mean	10.5	3.5	0.095
range	9.7-11.4	3.3-3.8	0.091-0.101
F_1 mean	10.7	3.5	0.102
range	9.6-11.4	2.9-4.0	0.098-0.108
F_2 mean	11.2	3.6	0.106
range	8.9-13.3	2.3-5.0	0.087-0.122
Bc_1 mean	11.4	3.7	0.110
range	8.9-13.3	2.3-5.3	0.094-0.122
Bc_2 mean	9.9	3.2	0.098
range	8.1-11.6	1.8-4.3	0.084-0.115

acids, and zein with valine, leucine and isoleucine contain a common element due to the relationship of a part with the whole. This can be illustrated by the case of protein and zein in the F_2 of Group I. Since zein constitutes 34.3 percent of the protein, the "spurious" correlation can be calculated (53) as $\rho = \sqrt{\frac{n_{12}}{n}}$, where n_{12} is the number of elements in a part, n is the number of elements in the whole, and ρ is the expected correlation. Substituting in this formula, $\rho = \sqrt{\frac{34.3}{100.0}} = .586$. To test the significance of the observed correlation coefficient, both it and the spurious correlation were transformed to "z values". This test had to be made because the tables for testing the significance of correlation coefficients compare the correlations with zero.

Since protein is composed primarily of amino acid residues, protein components should vary in the same direction. The fact that all of the observed correlation coefficients, given in Tables 6 through 11, are positive is in agreement with expectation.

The zein-protein correlations ranged from +0.76 to +0.97. They were all highly significant, which indicated that both the zein and non-zein portions increased simultaneously. Such high correlations also show that the observations involved fall very close to a straight line which describes the covariation of zein and protein percentages, or in other words, the variation in protein is closely related to the variation in zein.

With respect to valine and protein percentages, the correlations varied from +0.64 to +0.90 and all were significant. Those for leucine and protein, and isoleucine and protein ranged from +0.76 to +0.93, and

Table 6. Correlation coefficients involving the percentages of protein and certain protein components in the F_2 of Group I. (Observed "r" is lower number and spurious "r" is upper number)

	Zein %	Tryptophan %	Valine %	Leucine %	Isoleucine %
Protein %	.586 .969**	.095 .569**	.217 .900**	.385 .928**	.224 .903**
Zein %		- .534**	.156 .882**	.487 .888**	.207 .878**
Tryptophan %			- .559**	- .574**	- .502**
Valine %				- .828**	- .863**
Leucine %					- .716**

**Exceeds 1% level of significance

Table 7. Correlation coefficients involving the percentages of protein and certain protein components in the Bc_1 of Group I. (Observed "r" is lower number and spurious "r" is upper number)

	Zein %	Tryptophan %	Valine %	Leucine %	Isoleucine %
Protein %	.604 .959**	.089 .431*	.222 .843**	.312 .915**	.236 .840**
Zein %		- .405**	.156 .820**	.487 .884**	.207 .842**
Tryptophan %			- .424**	- .406**	- .401**
Valine %				- .766**	- .850**
Leucine %					- .719**

*Exceeds 5% level of significance

**Exceeds 1% level of significance

Table 8. Correlation coefficients involving the percentages of protein and certain protein components in the Bc_2 of Group I. (Observed "r" is lower number and spurious "r" is upper number)

	Zein %	Tryptophan %	Valine %	Leucine %	Isoleucine %
Protein %	.529 .926**	.102 .245	.224 .774**	.360 .763**	.229 .718**
Zein %		- .226*	.156 .728**	.487 .773**	.207 .693**
Tryptophan %			- .215*	- .285*	- .210*
Valine %				- .669**	- .596**
Leucine %					- .661**

*Exceeds 5% level of significance

**Exceeds 1% level of significance

Table 9. Correlation coefficients involving the percentages of protein and certain protein components in the F_2 of Group II. (Observed "r" is lower number and spurious "r" is upper number)

	Zein %	Tryptophan %	Valine %	Leucine %	Isoleucine %
Protein %	.567 .788**	.097 .332**	.228 .635**	.362 .804**	.243 .842**
Zein %		- .141	.156 .724**	.487 .739**	.207 .837**
Tryptophan %			- .206*	- .157	- .287*
Valine %				- .556**	- .726**
Leucine %					- .862**

*Exceeds 5% level of significance

**Exceeds 1% level of significance

Table 10. Correlation coefficients involving the percentages of protein and certain protein components in the Be_1 of Group II. (Observed "r" is lower number and spurious "r" is upper number)

	Zein %	Tryptophan %
Protein %	.570 .907**	.098 .412**
Zein %		- .373**

**Exceeds 1% level of significance

Table 11. Correlation coefficients involving the percentages of protein and certain protein components in the Be_2 of Group II. (Observed "r" is lower number and spurious "r" is upper number)

	Zein %	Tryptophan %
Protein %	.569 .756**	.099 .435**
Zein %		- .299**

**Exceeds 1% level of significance

+0.72 to +0.90, respectively. All of these are significant. Here, as in the case of zein and protein, the variation in protein is closely related to the variation in valine, leucine, and isoleucine.

The tryptophan-protein correlations ranged from +0.25 to +0.57, five of the six being significant. Although protein and tryptophan percentages are related positively, variations in the two are only slightly related. The correlations between zein and tryptophan ranged from +0.14 to +0.53, five of the six being significant. Since zein contains no tryptophan, here is a case of protein components of two different species increasing simultaneously. Because valine, leucine, and isoleucine constitute about 30 percent of zein, variation in zein percentage should be closely related to variation in any of the three. Correlations of zein with valine, leucine, and isoleucine which ranged from +0.72 to +0.88, from +0.74 to +0.89, and from +0.69 to +0.88, respectively, show this to be true. All of these correlations were significant.

The correlations of tryptophan percentage with valine, leucine, and isoleucine percentages indicated some relationship between tryptophan and the latter three. If valine, leucine, and isoleucine arise from a common precursor as their hydrocarbon moieties might lead one to suspect, they would be closely related. The correlations among them showed that a close relationship did exist. The correlations of valine with leucine, valine with isoleucine, and leucine with isoleucine ranged from +0.56 to +0.83, from +0.60 to +0.86, and from +0.66 to

0.86, respectively. All were highly significant.

Judging from the uniformity and magnitude of the correlation coefficients presented here, it may be concluded that the variation of protein, zein, valine, leucine, and isoleucine percentages in corn are all closely related. However, the variation of tryptophan percentage is only slightly related to the variation of any of the others.

The second method of studying the interrelationships of protein, zein, tryptophan, valine, leucine, and isoleucine percentages in corn, namely, the variation of protein components relative to protein variation, was accomplished by transforming the percentage data into logarithms and calculating "b" values for the following equation: $Y = aX^b$, in which Y is the expected value of the dependent variable, "a" is a constant, X is the independent variable, and "b" is the exponential regression of Y on X. If the dependent and the independent variables are present in the same relative amounts over the whole array of items, the value of "b" would be unity. Where protein and a protein component are independent and dependent variables, respectively, a value of "b" greater than 1.0 means that the protein component is a greater part of the protein in high than in low protein samples. For a value less than 1.0 the converse is true.

Table 12 gives the exponential regressions of each protein component upon total protein. The exponential regressions of zein percentage on protein percentage ranged from 1.44 to 1.95. All were highly significant when tested against unity. Therefore, zein makes up a

Table 12. Exponential regressions of certain protein component percentages on protein percentage in corn

Regression of:	Group I			Group II		
	F_2	Bc_1	Bc_2	F_2	Bc_1	Bc_2
Zein % on protein %	1.866**	1.558**	1.937**	1.427**	1.743**	1.873**
Tryptophan % on protein %	0.370**	0.306**	0.152**	0.272**	0.241**	0.363**
Valine % on protein %	0.934	0.782**	0.864	0.861	-	-
Isoleucine % on protein %	1.052	0.735**	0.893	1.055	-	-
Leucine % on protein %	1.296**	1.421**	1.074	1.147	-	-

**Significantly different from unity at 1% level

greater proportion of the total protein in high than in low protein samples. This fact, in turn, means that as higher protein percentage in corn is selected, the corn protein becomes less nutritionally balanced.

Knowing the zein-protein relationship, it would be expected that the regression of tryptophan on total protein would give an exponential value of less than one. The tryptophan-protein exponential regressions ranged from 0.15 to 0.37, all of which were significantly lower than unity.

Valine-protein exponential regressions ranged from 0.78 to 0.93 and only one was significantly lower than expected unity. Since all of the values were below 1.0, it was concluded that valine was not present in as large quantities relative to protein in high as in low protein samples. The exponential regressions of isoleucine on protein ranged from 0.74 to 1.06, and only one was significantly different from 1.0. Similar regressions for leucine on protein ranged from 1.07 to 1.42 with two of the four significantly greater than one. Since all of these values were above 1.0, leucine was present in slightly greater quantities relative to protein in high than in low protein samples.

Combining the information concerning the interrelationships of protein, zein, tryptophan, valine, leucine, and isoleucine percentages in corn obtained from both the correlationsand the exponential regressions, the following conclusions may be drawn:

1. The protein percentage and protein component percentages that were studied varied in the same direction simultaneously.

2. The variations of protein, zein, valine, leucine, and isoleucine percentages in the material studied were closely related to one another, but the tryptophan percentages were only slightly related to the others.

3. Zein became an increasing and tryptophan a decreasing proportion of the corn protein as protein percentage increased. Valine became a slightly decreasing, leucine a slightly increasing, and isoleucine a constant proportion of the protein as protein percent increased.

Total protein and non-zein protein percentages as measures of tryptophan content

Exponential regressions also give information concerning the hypothesis that non-zein-protein percentage is a better measure of tryptophan than is total protein percentage. Exponential regressions give a comparison of the relative increase of the dependent on the independent variable. From this it seemed logical that the variable with a rate of increase most like that of tryptophan was the better criterion for tryptophan selection. Then, by comparing the exponential regressions of tryptophan percentage on non-zein-protein and on protein percentages, it was possible to obtain information concerning which of these was a better criterion of tryptophan content. The comparisons are presented in Table 13.

Table 13. Exponential regressions contrasting total protein and non-zein-protein criteria of protein quality

	Population	Tryptophan % on protein %	Tryptophan % on non-zein protein %	Sign of difference (2) - (1)
	F_2	.370	.545	+
(Group I)	Bc_1	.306	.393	+
	Bc_2	.152	.222	+
	F_2	.272	.322	+
(Group II)	Bc_1	.241	.281	+
	Bc_2	.363	.409	+

The signs in column three all are positive, which means that in each of the six cases studied non-zein protein percentage is more closely related to tryptophan percentage than is total protein percentage. By applying the sign test of Dixon and Mood (15), this deviation (six pluses) from the expected (three minuses and three pluses) is found to be significant. This indicates that non-zein protein percentage is a better criterion of tryptophan content in corn than is total protein percentage.

Relation between protein percentage and weight of grain per plant

East and Jones (18) and Hayes and Garber (24) reported that protein percentage in corn was negatively correlated with amount of grain produced per plant. Also it is generally accepted that an increase in the yield of wheat or barley (19,31) is accompanied by a decrease in protein percentage.

In the present study the weights of grain per ear and the protein percentages were correlated. These correlations are given in Table 14.

Table 14. Correlation coefficients of weight of grain per ear with protein percentage

	Population	Correlation
(Group I)	F_2	-.483**
	Bc_1	-.360**
	Bc_2	-.222
(Group II)	F_2	-.099
	Bc_1	-.029
	Bc_2	-.265**

**Exceeds the 1% level of significance

All of the correlations were negative, and when the sign test was applied, it was found that the deviation from expected (three pluses and three minuses) was significant. These data indicate that protein percentage in corn is modified inversely by weight of grain produced per plant.

Effect of foreign pollen upon protein and tryptophan percentage

East and Jones (18) compared selfed ears with crossed ears in the same inbred lines of corn and found that those crossed had a significantly lower protein percentage than those selfed, but practically all of the reduction was accounted for by "heterotic dilution" of the protein. When crossed and selfed seeds were grown on the same ear, crossed kernels

Table 15. Comparison of protein and tryptophan in crossed and selfed kernels of corn

Sample number	Protein %			Tryptophan %			Mg. of protein per 10 kernels		Sign of S-C	Mg. of tryptophan per 10 kernels		Sign of S-C
	Selfed	Crossed	S-C	Selfed	Crossed	S-C	Selfed	Crossed		Selfed	Crossed	
1	19.3	18.0	0.7	0.106	0.093	0.013	495	504	-	2.72	2.60	+
2	18.3	17.8	0.5	0.118	0.106	0.012	372	374	-	2.40	2.23	+
3	16.2	15.9	0.3	0.118	0.090	0.028	354	372	-	2.58	2.11	+
4	18.3	15.8	2.5	0.094	0.088	0.006	401	366	+	2.06	2.04	+
5	19.0	18.3	0.7	0.104	0.104	0.0	438	455	-	2.40	2.59	-
6	13.8	14.9	-1.1	0.097	0.088	0.009	291	304	-	2.04	1.80	+
7	15.6	15.6	0.0	0.101	0.094	0.007	249	274	-	1.66	1.65	+
8	15.2	13.8	1.4	0.094	0.099	-0.005						
9	13.8	12.7	1.1	0.091	0.086	0.005	295	292	+	1.95	1.97	-
10	15.8	15.9	-0.1	0.094	0.086	0.008	343	371	-	2.04	2.01	+
11	17.1	16.2	0.9	0.114	0.102	0.012	413	425	-	2.75	2.67	+
12	19.0	19.3	-0.3	0.104	0.078	0.026						
13	13.0	12.5	0.5	0.110	0.099	0.011	289	291	-	2.44	2.31	+
14	18.2	16.7	0.5	0.110	0.099	0.011	390	350	+	2.36	2.08	+
15	16.3	14.2	2.1	0.104	0.090	0.014	361	325	+	2.30	2.06	+

actually contained slightly more protein by weight than selfed kernels. The same conclusions were drawn by Hayes and Garber (24). Hayes (23) recognized that the maternal plant determined the amount of protein in the grain.

In the present study, crossed and selfed seeds grown on the same ear were analyzed for protein and apparent tryptophan. The results of these analyses are given in Table 15. S-C refers to selfed minus crossed.

The "t" value for the mean difference in protein percentages between crossed and selfed was found to be 2.97, and for the same comparison upon tryptophan percentages 4.78. Both are highly significant. This indicates that crossing causes a reduction in protein and tryptophan percentages. In order to correct for the heterotic dilution of protein in the crossed seeds, weight of protein and tryptophan per ten kernels was calculated. The signs of the differences obtained by subtracting weight of protein per ten crossed seeds from weight of protein per ten selfed seeds were nine mimuses and four pluses. This is not a significant deviation from expected, which means that the lower protein percentage due to crossing was primarily due to heterotic dilution. In the same test for tryptophan the signs of the differences were eleven pluses and two mimuses. This may be a significant deviation from expected. If so, the absolute amount of tryptophan was greater in selfed than in crossed kernels.

This section emphasizes the need for selfing when undertaking a study on the inheritance of protein or protein component percentages in corn.

Yearly variation of tryptophan and protein percentages

The protein percentages for six inbreds grown from 1942 through 1945 and the analysis of variance of the data are given in Table 16. There were no significant differences among years, but the differences among lines were significant.

Tryptophan percentages for eight inbreds grown from 1943 through 1946 with the accompanying analysis of variance are given in Table 17. Year and line differences were significant.

Protein, zein, and tryptophan percentages of Corn Belt inbreds

Sixty-four Corn Belt inbreds grown in 1946 were analyzed for protein, zein, and tryptophan. The results of these analyses are given in Table 18.

Protein varied from 9.0 to 14.7 percent with the majority of the inbreds falling between 10.0 and 11.5. Inbred B_2, which was highest in protein percentage, also was high in tryptophan percentage. L289 and Ia153 had a low zein-protein ratio. Protein and zein percentages were 12.4 and 3.1, respectively, for Ia153, and 10.7 and 2.6, respectively, for L289. In order to determine whether the favorable protein-zein balance in these two inbreds was inherent or environmental, samples of both grown in other years were analyzed. The favorable balance observed in L289 was environmental, while that if Ia153 was inherent.

Tryptophan percentages ranged from 0.084 to 0.118 percent. Inbred 187-2 was outstanding with 0.118 percent tryptophan.

Table 16. Yearly variation in protein percentages of six inbred lines

Inbred	1942	1943	1944	1945	Total	Average
L317	12.6	11.1	11.9	11.7	47.3	11.9
Ky	10.6	10.5	11.4	10.5	43.0	10.8
K1241	11.3	11.7	11.1	12.1	46.2	11.5
Y63	12.1	10.6	10.4	12.4	45.5	11.4
Oh51A	11.7	11.7	11.9	11.2	46.5	11.6
L289	12.3	12.1	12.3	12.8	49.5	12.4
Total	70.6	67.7	69.0	70.7	278.0	

Analysis of variance

Variance due to	DF	MS
Total	23	
Years	3	.34
Lines	5	1.14*
Y x L	15	.33

*Significant at the 5% level

Table 17. Yearly variation in the tryptophan percentage of eight inbred lines

Inbred	1943	1944	1945	1946	Total	Average
Oh28	.086	.091	.096	.096	.369	.092
L317	.094	.096	.096	.096	.382	.095
Hy	.096	.096	.096	.096	.384	.096
B3	.096	.096	.099	.096	.387	.097
K1241	.096	.103	.094	.107	.400	.100
Y63	.096	.103	.107	.107	.413	.103
Oh51A	.107	.103	.107	.107	.424	.106
L289	.113	.113	.120	.120	.466	.116
Total	.784	.801	.815	.825	3.225	

Analysis of variance

Variance due to	DF	MS
Total	31	
Years	3	.000039*
Lines	7	.000240**
L x Y	21	.000010

*Significant at the 5% level

**Significant at the 1% level

Table 18. Protein, zein, and tryptophan percentages of sixty-four inbreds grown in 1946

Inbred	Protein %	Zein %	Tryptophan %
B2	14.7	6.7	0.115
B3	10.2	3.3	0.084
B4	9.0	2.8	0.084
B5	13.1	4.5	0.101
B6	10.9	3.7	0.094
B7	11.1	3.7	0.091
B4-604	12.4	5.0	0.111
B4-668	11.4	4.4	0.105
Oh28	9.5	2.5	0.084
Oh51A	9.8	2.8	0.094
Oh40B	10.2	3.5	0.091
Oh07	10.5	3.5	0.101
Oh67A	11.9	4.0	0.111
M14	11.0	3.5	0.108
M8-29	12.1	4.5	0.091
M1984	10.5	3.6	0.101
EK1969	10.0	3.4	0.108
SSS278	10.2	3.0	0.098
SSS211	11.4	4.1	0.111
I233	11.9	5.6	0.084
I198	11.4	3.9	0.108
I159	10.5	3.2	0.094
Ia153	12.4	3.1	0.105
I159L1	11.2	3.5	0.091
I205	11.0	3.9	0.091
I224	11.5	4.0	0.091
L234	10.1	2.9	0.101
L289	10.7	2.6	0.108
L317	10.9	3.4	0.084
I224R4	10.5	3.7	0.108
I205 (S_2)	12.4	4.7	0.084
Y31	10.5	2.9	0.108
Y63	10.1	3.6	0.098
Y82	12.1	3.9	0.101
I205	12.6	4.4	0.101

Table 18. (Cont'd)

Inbred	Protein %	Zein %	Tryptophan %
B1345	9.7	2.7	0.084
B1349	11.4	3.8	0.098
B1351	11.5	4.2	0.098
K1409	10.7	3.7	0.101
K1309	10.1	2.8	0.101
K1241	10.1	3.1	0.094
Kr1903	11.6	4.5	0.108
Kr1921	10.0	3.3	0.108
K155	11.9	4.7	0.084
CC5	9.5	2.3	0.087
CC7	13.0	4.0	0.111
CC28	11.2	3.7	0.098
C1447	10.9	3.8	0.098
Hy	10.3	3.3	0.084
WF9	12.1	4.3	0.111
(WF9xD17)561	10.5	3.1	0.111
R4	11.6	3.8	0.105
Tr	11.0	3.7	0.094
187-2	10.2	3.7	0.118
38-11	12.8	5.5	0.101
507-193	9.5	2.9	0.087
ITE701	9.1	2.6	0.094
Os426	11.4	4.3	0.101
Os420	11.9	4.4	0.105
Mo401	11.1	3.8	0.087
A	11.2	3.7	0.098

Discussion

So far as the writer can determine, no statistical studies have been reported on the relationship between protein and any of its component amino acids in corn. However, Hansen, Brimhall, and Sprague (22) studied the relationship between zein, the alcohol soluble fraction, and total protein percentages, and found a correlation coefficient of +0.92. This same close relationship is shown in the present study by the fact that four of the six correlations reported between these two variables were above +0.90 and the other two were above +0.75. Further evidence that zein and protein are closely related is the fact that only four samples of 700 analyzed have deviated far enough from regression in the favorable direction to warrant selection.

The data of Showalter and Carr (52) indicated that the zein and non-zein portions of corn protein did not increase at the same relative rate when protein percentage was increased. The data of the present study are in good agreement with this last point, as evidenced by the exponential regressions of zein percentage on protein percentage which are all significantly greater than one. As a sequel to the zein-protein relationship, the exponential regressions of tryptophan on protein were all significantly less than one.

All of the amino acids, tryptophan, valine, leucine, and isoleucine were correlated positively and significantly with one another. This is evidence that all of them increased simultaneously. However, it must be

remembered that these correlations were made on samples with a wide range of protein percentages, and that in selection for one amino acid at a constant protein percentage, the relation between it and other amino acids probably will be negative unless the two being correlated are of the same species. The fact that all of the valine-protein exponential regressions were less than unity can be explained by the fact that zein does not contain as high a proportion of valine as does non-zein protein. Since increasing protein percentage is accompanied by a disproportionate increase in zein, valine is not expected to keep pace with protein. This point is of academic interest and not of practical importance in corn protein quality, because even in high protein corns, valine is present in copious amounts relative to lysine and tryptophan.

Two schools of thought prevail with regard to the best criterion for selection of improved protein quality in corn. One school maintains that selection for high protein percentage would result in an improvement of the feeding value of corn. This statement is granted. However, it has been shown that yield of barley and wheat was negatively correlated with protein percentage, and data have been given in the present study which indicated a similar relationship in corn. Thus, when selection is made for high protein percentage, a corresponding decrease in yield will occur, and the production of protein, and what is more important, tryptophan per acre, will not have increased. The persons supporting this school of thought will obtain by their method

of selection lower yields of corn with higher protein content; however, the corn protein will be of poorer quality. The writer maintains that selection only for high protein percentage will not accomplish the ultimate objective, which is a corn with yields as great as those obtained today, and with yields of tryptophan much greater than those obtained today. Selection should be for a corn with nine to ten percent protein, of which the protein contains a much higher proportion of tryptophan than any analyzed to date. A corn of this type may yield well and thus produce much more tryptophan per unit of land area.

The second school of thought holds that selection for a medium protein percentage with a low proportion of zein in the protein is the most expedient method of improving corn protein quality. This procedure will increase the non-zein proportion of protein and thus increase tryptophan content.

A comparison of these two methods was presented in Table 13. The sign test applied to this data indicated that non-zein protein was a better criterion of tryptophan content than total protein.

Furthermore, selecting corn with a low zein-protein ratio will emphasize selection for improved quality in endosperm protein. This is in contrast to the method where quality is improved by selection on the basis of tryptophan percentage only. The selection of high tryptophan percentage will tend to lead in two directions, neither of which is the desired objective: (1) The increased tryptophan percentage will be accomplished in part by selection for germ protein. Germ size is readily modified while the normal zein-protein balance is apparently

very stable. Since germ protein contains relatively large amounts of tryptophan, the corn selected will contain a large germ with relatively little improvement in endosperm protein. Illinois high oil corn exemplifies this situation. It has about twenty to twenty-five percent germ and the highest tryptophan percentage of any corn analyzed to date. (2) Tryptophan percentage is positively correlated with protein percentage, so the resulting selections will tend to have a high protein percentage, and this situation is disadvantageous for reasons given above.

Both of the latter methods of selection for increased tryptophan content are now being used at the Iowa Experiment Station in conjunction with the truncation system of breeding. No data are yet available to evaluate the two methods.

It is the writer's conviction that the best procedure will be a combination of the two methods, whereby tryptophan analyses are made and then the endosperm of the high tryptophan samples analyzed for zein-protein balance.

The negative correlations between yield of grain per plant and protein percentage found in this study are in good agreement with similar studies in wheat and barley (19, 31). East and Jones (18) and Hayes (23) found a negative correlation between number of seeds per ear and the protein percentage of the grain.

A critical test to prove the relationship between yield and protein percentage in corn is now possible on a field plot basis rather than on an individual plant basis, since commercial companies have hybrid corns

which contain 14-15 percent protein. To do this a replicated yield trial, including adapted corns with a wide range of protein percentage, should be planted. Reduction of protein content due to open pollination would not be a factor under such a scheme. After yield data are obtained, the corn could be analyzed for protein. Correlations between protein percentage and yield of corn grown under the suggested set of conditions would suffice to prove or disprove the contention that these two variables are inversely related.

Another study which should be made along this line is the effect of nitrogen fertilization upon protein percentage and composition. This could be done by growing corns with different protein percentages at varying levels of nitrogen. It may be found in such a test that the inverse relation between yield and protein percentage is only an expression of limited nitrogen supply.

In regard to heterotic dilution of protein percentage, the data in the present study are in very good agreement with those of East and Jones (18) and Hayes (23). This phenomenon probably is the result of limited nitrogen supply without a corresponding limited supply of carbon dioxide.

Summary

Between eighty and one hundred individual ear samples of each of the F_2, Bc_1, and Bc_2 of a cross between Illinois high and low protein corns were analyzed for protein, zein, tryptophan (the tryptophan analysis procedure may give only relative results), valine, leucine, and isoleucine. Protein, zein, and tryptophan analyses were made on comparable material from the cross Hy x Il98, and valine, leucine, and isoleucine analyses were made on every third sample of the F_2. From these data it was concluded that:

1. All of the protein components studied varied simultaneously and in a positive manner.

2. Zein became a greater and tryptophan a lesser proportion of protein as protein percentage increased. It was concluded that selection for higher protein percentage would likely be accompanied by a decrease in the quality of corn protein. Furthermore, valine, leucine, and isoleucine percentages increased at approximately the same rate as protein percentage.

3. The variations in protein, zein, valine, leucine, and isoleucine percentages were very closely related to one another, while variation in tryptophan content was only slightly related to variation in any of the others.

4. Evidence was given to show that non-zein protein percentage would be a better criterion of selection for increased tryptophan

content in corn than total protein percentage. In the F_2, Be_1, and Be_2 of both groups, a comparison of these two criteria of selection showed non-zein protein percentage to be the better.

It was pointed out that the ultimate procedure for improving protein quality probably will be to analyze for tryptophan percentage and then analyze the highest tryptophan samples for zein-protein relationship. Only those samples which are high in tryptophan due to a favorable zein-protein relationship should be saved for future breeding work.

5. The weight of grain per ear was correlated with protein percentage. In each of the six populations, the correlation was negative.

6. The crossed kernels on Illinois high protein ears contained a significantly lower protein percentage than the selfed kernels on the same ear, but this reduction could be accounted for by heterotic dilution.

7. In a similar comparison, tryptophan percentage was significantly less in the crossed than in the selfed kernels.

8. Six inbreds grown in each of four years were analyzed for protein. This data showed significant differences among lines, but not among years.

9. Eight inbreds grown in each of four years were analyzed for tryptophan. This data showed significant differences among lines and among years.

10. Sixty-four widely used Corn Belt inbreds grown in 1946 were analyzed for protein, zein and apparent tryptophan. Ia153 had a low percentage of zein in relation to its protein percentage. It also was among the inbreds with high tryptophan content. The inbred 187-2 was highest in tryptophan percentage, and B_2 was highest in protein percentage.

PART II. INHERITANCE OF PROTEIN AND SOME OF ITS COMPONENTS

Review of Pertinent Literature

The first extensive protein selection experiments in corn were inaugurated at the Illinois Experiment Station in 1896. High protein selection was started to produce a good yielding, high protein variety of corn. To test the effectiveness of the selection methodology, low protein strains were selected simultaneously. The experiments were begun in Burr's dent corn by selecting from 164 ears that averaged 10.9 percent protein the 24 with the highest and the 12 with the lowest protein percentages. Since 1896 ear-to-row selection, with slight modifications, has been practiced on both protein strains. Hopkins (26) reported in 1898 that a noticeable shift had occurred in mean protein percentage in both strains. In later papers (27, 59) it was reported that protein percentage in the low selections had apparently come to a lower limit, but protein percentage in the high selections was still increasing slightly.

East and Jones (18) started a program similar to the one in Illinois except that they used straight selfing as a means for isolation of high protein strains. By this method both paternal and maternal germ plasm were controlled, while in ear-to-row selection, at least during the early stages, only the maternal germ plasm was effectively selected.

East and Jones (18) made as much progress in six generations of selection as the workers at Illinois did in fifteen. In this paper data were presented to evaluate a new method of isolating inbred lines of corn superior in protein percentage. Today this method is called "recurrent selection". Their data showed that rapid progress could be made with this system, which consisted of crossing superior strains and selecting within the intercross population. Hayes and Garber (24) also proposed this method. The progress made in the selection experiments at Illinois and Connecticut offered proof that protein percentage in corn was inherited. Arbuckle and Thies (1) concluded that heredity was practically the sole source of variation of protein percentage in corn.

East and Jones (18) presented further data on the inheritance of protein percentage in a series of F_1 hybrids, from which they concluded first, that low protein percentage was partially dominant, and second, that a great number of genes determined protein percentage. In contrast to the dominance of low protein found by East and Jones, Hayes (23) produced a double cross from high protein inbreds which yielded 15.0 percent protein in a year when Minnesota 13 yielded only 12.4 percent. Later Lindstrom and Gerhardt (32) reported on a cross of high oil x Evergreen sweet corn in which the F_2 generation corresponded to the F_1 generation in the present study. With this point in mind, their data are interpreted as showing dominance of low protein percentage.

Inheritance of protein percentage in wheat was studied by Clark (12), Clark and Hooker (13), and Clark and Quisenberry (14) in the F_2's

and F_3's of several crosses. Low protein tended to be dominant. This was shown by skewness of F_2 populations and also by the fact that F_3 progenies from low protein F_2 individuals showed more variation than those from high protein F_2 individuals.

To the best of the writer's knowledge, only one study has been made on the inheritance of individual amino acids, and this in corn by Doty, et al. (17). They analyzed 28 single crosses grown in 1939 and 1940 for protein, tryptophan, tyrosine, histidine, cystine, and arginine, and despite large fluctuations, their data showed that protein character was controlled somewhat by heredity. In 1940 and 1941 they analyzed all possible single crosses among L317, Hy, 38-11, WF9, 187-2, and R4 in the midseason group, and Hy, Mo940, 38-11, Mo824, L317, and K4 in the late group. By averaging the protein percentage of all crosses involving an inbred, identical to evaluating general combining ability, it was possible to index the inbred's protein-producing potentialities. This was done for each inbred. Inbred 38-11 gave high and L317 gave low protein percentages in their respective single crosses. Crosses with Hy and L317 contained the highest percentage of tryptophan, and Hy, WF9, 187-2, and R4 in crosses were high in tyrosine percentage. Histidine percentage did not vary appreciably. L317 and 38-11 in crosses were high in cystine percentages, and K4 was outstanding in arginine percentage. They concluded that amino acids in corn were under genetic control and therefore protein quality could be improved.

Studies upon the nature of the interaction of genes determining

yield have been made in Zea mays and Lycopersicum species. MacArthur and Butler (34) and Powers (50) presented data on fruit size in tomato crosses which conformed very well to expected values based upon the hypothesis of geometric gene interaction. In a second paper by Powers (51) on fruit size in a tomato cross, neither the expected values for geometric or arithmetic gene interaction fit the observed data.

In corn, Kinman and Sprague (29) found that the type of gene interaction for yield was approximated more closely by arithmetic than by geometric gene interaction. Likewise, Neal (46) reported that the reduction in yield in the second generation of single, double, and three-way crosses of corn was very close to that expected upon the hypothesis of arithmetic gene interaction.

Mangelsdorf and Fraps (37) and Fraps (20), making use of the well known fact that yellow endosperm is very closely associated with vitamin A content, studied the nature of intra-allelic gene action in corn endosperm. By controlled crossing between yellow and white strains of corn, it was possible to obtain endosperm containing zero, one, two, and three genes for yellow color. They found that the yellow endosperm genes acted in an arithmetic cumulative manner with each gene adding 2.5 units of vitamin A per gram of corn.

Materials and Methods

For a description of materials and methods see pages 8 to 17.

Experimental Results

Dominance

The mean percentages of protein, zein, tryptophan, valine, leucine, and isoleucine for the cross Illinois high x Illinois low protein are given in Table 19, and the mean percentages of protein, zein, and tryptophan for the cross Hy x H198 are given in Table 20. The frequency distributions for these variables are shown in Figures 3 through 12 in the appendix.

Protein percentage. In neither cross was the F_1 mean protein percentage significantly different from the mean of the lower protein parent. The mean protein percentages of the Bc_2's were lower than their respective lowest protein parents. In each case, the F_2 mean was substantially larger than the F_1 mean but did not reach the midpoint between the parents. The consistency of the data in both crosses leads the writer to the conclusion that low protein percentage is completely dominant.

Zein percentage. Zein percentage behaved similarly to protein percentage. The F_1 zein percentage means were equal to or slightly below their respective P_2 means. The Bc_2 means were slightly below their respective P_2 means. In both groups, the F_2 mean was between the two backcrosses and did not approach the midpoint between the parents. From these data it appears that low zein percentage is completely dominant.

Table 19. Mean percentages of protein, zein, tryptophan, valine, leucine, and isoleucine for Group I (Illinois high x Illinois low protein)

	Protein %	Zein %	Tryptophan %	Valine %	Leucine %	Isoleucine %
P_1	17.2	7.2	0.122	0.83	2.99	0.98
P_2	7.2	2.1	0.084	0.34	0.79	0.39
F_1	6.6	1.7	0.074	0.34	0.96	0.32
F_2	9.6	3.3	0.087	0.45	1.42	0.48
Bc_1	11.8	4.3	0.094	0.58	1.72	0.66
Bc_2	6.8	1.9	0.072	0.34	0.88	0.42

Table 20. Mean percentages of protein, zein, and tryptophan for Group II (My x I198)

	Protein %	Zein %	Tryptophan %
P_1	12.8	4.9	0.114
P_2	10.5	3.5	0.095
F_1	10.7	3.5	0.102
F_2	11.2	3.6	0.106
Bc_1	11.4	3.7	0.110
Bc_2	9.9	3.2	0.098

Tryptophan percentage. In the high x low protein cross, the F_1 mean tryptophan percentage was decidedly lower than that of the low protein parent. The Bc_2 mean was lower than either the F_1 or P_2. The F_2 was slightly higher than the P_2 mean, and the Bc_1 mean did not approach the midpoint between the two parents. Besides showing complete dominance of low tryptophan percentage, the phenomenon of heterotic dilution probably displays its greatest effect here.

The F_1 mean of Group II was intermediate between those of the parents, and that of the Bc_2 was half way between the F_1 and P_2 means. Apparently no dominance is present with respect to tryptophan percentage in the Hy x I198 cross.

The results from the two crosses differed in respect to dominance in tryptophan percentage. The high x low protein cross showed complete dominance of low tryptophan percentage, while the Hy x I198 cross showed no dominance.

Valine, leucine, and isoleucine percentages. The inheritance of valine, leucine, and isoleucine percentages was studied in only the high x low protein cross. The F_1 and Bc_2 mean valine percentages were exactly the same as the P_2 mean. This is indicative of complete dominance of low valine percentage.

The mean leucine percentage of the F_1 was higher than the low protein parent mean and the Bc_2 was half way between the F_1 and P_2. The F_2 mean did not reach the midpoint between the two parents. It seems quite safe to say that low leucine percentage is partially dominant.

Isoleucine percentage behaved similarly to protein percentage. The F_1 mean was lower than that of the P_2, the Bc_2 mean was slightly higher than that of the P_2, and the F_2 mean did not reach the midpoint between the parents. The deduction that low isoleucine percentage is completely dominant seems valid.

Nature of interaction of genes

Formulae are available for computing the expected means of quantitative characters in F_2, Bc_1, and Bc_2 populations on the assumption of arithmetic or geometric gene interaction. These were given by Smith and Charles (11). The formulae for computing the expected means and their respective variances on the assumption of arithmetic gene interaction are as follows:

$$(1)\quad E(\bar{x}_{F_2}) = \frac{\bar{x}_{P_1} + 2\bar{x}_{F_1} + \bar{x}_{P_2}}{4}$$

$$(1a)\quad V\left[E(\bar{x}_{F_2})\right] = \frac{s^2_{\bar{x}P_1} + 4s^2_{\bar{x}F_1} + s^2_{\bar{x}P_2}}{16}$$

$$(2)\quad E(\bar{x}_{Bc_1}) = \frac{\bar{x}_{P_1} + \bar{x}_{F_1}}{2}$$

$$(2a)\quad V\left[E(\bar{x}_{Bc_1})\right] = \frac{s^2_{\bar{x}P_1} + s^2_{\bar{x}F_1}}{4}$$

$$(3)\quad E(\bar{x}_{Bc_2}) = \frac{\bar{x}_{P_2} + \bar{x}_{F_1}}{2}$$

$$(3a)\quad V\left[E(\bar{x}_{Bc_2})\right] = \frac{s^2_{x_{P_2}} + s^2_{x_{F_1}}}{4}$$

In all of the formulae E is the expected value, V is variance of an expected value, s^2 is the sample variance, and $\bar{x}$ is the sample mean. The corresponding formulae for computing expected means on the assumption of geometric gene interaction are:

$$(1)\quad E(\bar{x}_{F_1}) = \sqrt{\bar{x}_{P_1} \cdot \bar{x}_{P_2}}$$

$$(2)\quad E(\bar{x}_{F_2}) = \bar{x}_{F_1}\ 1 + \frac{1}{3n}(\log \bar{x}_{P_1} - \log \bar{x}_{P_2})^2$$

$$(3)\quad E(\bar{x}_{Bc_1}) = \sqrt{\bar{x}_{P_1} \cdot \bar{x}_{F_2}}$$

$$(4)\quad E(\bar{x}_{Bc_2}) = \sqrt{\bar{x}_{P_2} \cdot \bar{x}_{F_2}}$$

Formulae for computing the variances of expected means upon the hypothesis of geometric gene interaction were not available, and since "t tests" were desired for observed vs. geometric comparisons, variances of the expected means were essential. Therefore, the formulae were modified to a form for which variances were computable. The modified formula presented below gives a very close approximation to the one for geometric gene interaction:

$$E(\bar{x}_{\log F_1}) = \frac{\bar{x}_{\log P_1} + \bar{x}_{\log P_2}}{2}$$

$$V\left[E(\bar{x}_{\log F_1})\right] = \frac{s^2_{\bar{x}_{\log P_1}} + s^2_{\bar{x}_{\log P_2}}}{4}$$

The same modification was made for the backcross formulae. It was impossible to convert the F_2 formula to a form for which a variance was available.

The comparisons of Group I observed means with means calculated on the assumption of arithmetic and geometric gene interaction are given for protein, zein, tryptophan, valine, leucine, and isoleucine percentages in Tables 21, 22, 23, 24, 25, and 26, respectively, and for Group II, protein, zein,and tryptophan percentages in Tables 27, 28, and 29, respectively.

The observed protein percentages in Group I fit those calculated on the assumption of arithmetic gene interaction, while in Group II the observed means did not fit those calculated on the assumption of either arithmetic or geometric gene interaction. The zein data behave in a manner similar to the protein data. Group I observed zein percentage means conformed to those calculated on the basis of arithmetic gene interaction. For Group II, the zein means calculated for arithmetic and geometric gene interaction were so much alike that it was impossible to tell to which scheme, if either, the observed data conformed.

Group I observed amino acid means conformed to those calculated

Table 21. Comparison of observed protein percentage means in Group I with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical protein % means		Observed protein % means	Arithmetic vs. observed			Theoretical mean of protein % logs	Observed mean of protein % logs	Log vs. observed[a]		
	Arithmetic	Geometric		Difference	S_d[b]	t			Difference	S_d[b]	t
P_1			17.2					1.2341			
P_2			7.2					0.9485			
F_1		11.1	6.6				1.0913	0.8046	0.2867	0.091	3.12
F_2	9.4	6.7	9.6	-0.2	0.324	0.62		0.9708			
Bc_1	11.9	12.8	11.8	0.1	0.384	0.26	1.1038	1.0632	0.0406	0.011	3.69
Bc_2	6.9	8.3	6.8	0.1	0.302	0.33	0.9610	0.8290	0.1320	0.012	11.00

[a]In reality, a test of geometric vs. observed

[b]Standard error of the difference

Table 22. Comparison of observed zein percentage means in Group I with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical zein % means		Observed zein % means	Arithmetic vs. observed			Theoretical mean of zein % logs	Observed mean of zein % logs	Log vs. observed[a]		
	Arithmetic	Geometric		Differences	S_d[b]	t			Difference	S_d[b]	t
P_1			7.4					0.8737			
P_2			2.1					0.3230	0.4100	0.056	7.32
F_1		3.9	1.7				0.5984	0.1884			
F_2	3.2	1.7	3.3	-0.1	0.188	0.56		0.4776			
Be_1	4.6	4.9	4.3	0.3	0.228	1.32	0.6757	0.6204	0.0553	0.017	3.25
Be_2	1.9	2.6	1.9	0.0	0.170	0.0	0.4003	0.2782	0.1221	0.026	4.70

[a]In reality, a test of geometric vs. observed

[b]Standard error of the difference

Table 23. Comparison of observed tryptophan percentage means in Group I with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical tryptophan % means		Observed tryptophan % means	Arithmetic vs. observed			Theoretical mean of tryptophan % logs	Observed mean of tryptophan % logs	Log vs. observed[a]		
	Arithmetic	Geometric		Difference	S_d[b]	t			Difference	S_d[b]	t
P_1			0.122					-0.9178			
P_2			0.084					-1.0703			
F_1		0.101	0.074				-0.9940	-1.1344	0.1404	0.031	4.53
F_2	0.088	0.075	0.087	0.001	0.0022	0.45		-1.0669			
Bc_1	0.098	0.103	0.094	0.004	0.0023	1.74	-0.9923	-1.0341	0.0418	0.007	5.97
Bc_2	0.079	0.086	0.072	0.007	0.0030	2.33	-1.0686	-1.1490	0.0804	0.029	2.77

[a] In reality, a test of geometric vs. observed

[b] Standard error of the difference

Table 24. Comparison of observed valine percentage means in Group I with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical valine % means		Observed valine % means	Arithmetic vs. observed			Theoretical mean of valine % logs	Observed mean of valine % logs	Log vs. observed[a]		
	Arithmetic	Geometric		Difference	S_d[b]	t			Difference	S_d[b]	t
P_1			0.83					-0.0802			
P_2			0.34					-0.4866			
F_1		0.53	0.34				-0.2834	-0.4893	0.2059	0.033	6.24
F_2	0.46	0.34	0.45	0.01	0.017	0.59		-0.3572			
Bc_1	0.59	0.61	0.58	0.01	0.019	0.53	-0.2187	-0.2404	0.0217	0.011	1.97
Bc_2	0.34	0.39	0.34	0.0	0.017	0.0	-0.4219	-0.4810	0.0591	0.013	4.55

[a]In reality, a test of geometric vs. observed

[b]Standard error of the difference

Table 25. Comparison of observed leucine percentage means in Group I with expected means calculated for arithmetic and geometric gene interaction

Material	Theoretical leucine % means		Observed leucine % means	Arithmetic vs. observed			Theoretical mean of leucine % logs	Observed mean of leucine % logs	Log. vs. observed[a]		
	Arithmetic	Geometric		Difference	S_d[b]	t			Difference	S_d[b]	t
P_1			2.99					0.4660			
P_2			0.79					-0.1031			
F_1		1.53	0.96				0.1815	-0.0315	0.2130	0.099	2.15
F_2	1.43	0.97	1.42	0.01	0.066	0.15		0.1309			
Bc_1	1.98	2.06	1.72	0.26	0.092	2.83	0.2985	0.2152	0.0833	0.019	4.38
Bc_2	0.88	1.06	0.88	0.0		0.0	0.0139	-0.0637	0.0776	0.018	4.31

[a] In reality, a test of geometric vs. observed

[b] Standard error of the difference

Table 26. Comparison of observed isoleucine percentage means in Group I with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical isoleucine % means		Observed isoleucine % means	Arithmetic vs. observed			Log vs. observed[a]				
	Arithmetic	Geometric		Difference	S_d[b]	t	Theoretical mean of isoleucine % logs	Observed mean of isoleucine % logs	Difference	S_d[b]	t
P_1			0.98					-0.0142			
P_2			0.39					-0.4173			
F_1		0.62	0.32				-0.2158	-0.5000	0.2842	0.029	9.80
F_2	0.50	0.33	0.48	0.02	0.018	1.10		-0.3295			
Bc_1	0.65	0.69	0.66	-0.01	0.022	0.45	-0.1718	-0.1892	0.0174	0.011	1.58
Bc_2	0.36	0.43	0.42	-0.06	0.017	3.58	-0.3734	-0.3936	0.0202	0.015	1.35

[a]In reality, a test of geometric vs. observed

[b]Standard error of the difference

Table 27. Comparison of observed protein percentage means in Group II with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical protein % means		Observed protein % means	Arithmetic vs. observed			Theoretical mean of protein % logs	Observed mean of protein % logs	Log vs. observed[a]		
	Arithmetic	Geometric		Difference	S_d[b]	t			Difference	S_d[b]	t
P_1			12.8					1.1040			
P_2			10.5					1.0199			
F_1		11.6	10.7				1.0620	1.0297	0.0323	0.0053	6.09
F_2	11.2	10.8	11.2	0.0	0.118	0.0		1.0474			
Bc_1	11.7	11.9	11.4	0.3	0.121	2.47	1.0757	1.0544	0.0213	0.0045	5.73
Bc_2	10.6	10.8	9.9	0.7	0.128	5.46	1.0337	0.9936	0.0401	0.0042	9.55

[a]In reality, a test of geometric vs. observed

[b]Standard error of the difference

Table 28. Comparison of observed zein percentage means in Group II with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical zein % means		Observed zein % means	Arithmetic vs. observed			Theoretical mean of zein % logs	Observed mean of zein % logs	Log vs. observed[a]		
	Arithmetic	Geometric		Differences	S_d[b]	t			Difference	S_d[b]	t
P_1			4.9					0.6883			
P_2			3.5					0.5466			
F_1		4.1	3.5				0.6175	0.5467	0.0708	0.0110	6.44
F_2	3.8	3.5	3.6	0.2	0.073	2.74		0.5552			
Bc_1	4.2	4.2	3.7	0.5	0.069	7.25	0.6217	0.5602	0.0615	0.0099	6.21
Bc_2	3.5	3.5	3.2	0.3	0.088	3.41	0.5509	0.4878	0.0631	0.0083	7.60

[a]In reality, a test of geometric vs. observed

[b]Standard error of the difference

Table 29. Comparison of observed tryptophan percentage means in Group II with expected means calculated for arithmetic and geometric gene interaction

Materials	Theoretical tryptophan % means		Observed tryptophan % means	Arithmetic vs. observed			Theoretical mean of tryptophan % logs	Observed mean of tryptophan % logs	Log vs. observed[a]		
	Arithmetic	Geometric		Difference	S_d[b]	t			Difference	S_d[b]	t
P_1			0.114					-0.9438			
P_2			0.095					-1.0230			
F_1		0.104	0.102				-0.9834	-0.9946	0.0112	0.0051	2.20
F_2	0.103	0.102	0.106	0.003	0.0009	3.33		-0.9772			
Be_1	0.108	0.110	0.110	0.002	0.0007	2.82	-0.9605	-0.9593	-0.0012	0.0036	0.33
Be_2	0.099	0.100	0.098	0.001	0.0011	0.94	-1.0001	-1.0093	0.0092	0.0036	2.56

[a]In reality, a test of geometric vs. observed

[b]Standard error of the difference

upon the assumption of arithmetic gene interaction very well with the exception of tryptophan in the Bc_2, leucine in the Bc_1, and isoleucine in the Bc_2. There was a significant disparity between the observed data and those calculated on the assumption of geometric gene interaction in each case except isoleucine in the Bc_1 and Bc_2, and valine in the Bc_1. In Group II, it was impossible to determine whether the observed tryptophan means conformed better to those calculated on the assumption of geometric or arithmetic gene interaction because calculated means for both schemes were so nearly identical.

Gene number

Estimating the number of genes affecting a quantitative character is a controversial issue, and any results on the subject should be viewed with reservations. The cause of certain unreliabilities in estimated gene number is not that the formulae used are inaccurate, but rather that the assumptions underlying the formula are not fulfilled by the experimental material. The formula used for calculating gene number was derived by Castle (9 and 10). It is as follows:

$$n = \frac{(F_2 \text{ range})^2}{8(s_{F_2} - s_{F_1}{}^2)}$$

where n is the number of gene pairs, F_2 range is the highest F_2 individual minus the lowest F_2 individual, $s_{F_2}{}^2$ is the variance of the F_2, and $s_{F_1}{}^2$ is the variance of the F_1.

The assumptions underlying this formula are:

1. All genes act in an additive manner.
2. The poor parent contains only poor genes, and the good parent contains only good genes for the character in question.
3. All genes have equal effects.
4. The F_1 variance represents only environmental variance.
5. No dominance is present.

In the cross of Illinois high and low protein corns, it has been shown that the genes determining protein and amino acid percentages interact in an additive manner, so assumption number one is fulfilled. Also, it was pointed out that these strains appear to be asymptotic to a ceiling and a floor, respectively. Probably this floor and ceiling represent the minimum and maximum protein percentages which can be attained by selection acting upon the genes present in the original Burr White selections. Then within the genetic limits of the original selections, the high protein strain contains a majority of genes favorable for high protein percentage and very few favorable for low protein percentage. The converse is true for low protein corn. Thus assumption two is reasonably well fulfilled. However, assumptions three, four, and five probably are not. Since zein, valine, leucine and isoleucine parallel protein, it seems safe to say that assumptions one and two are fulfilled by data on them. The genes determining tryptophan interact arithmetically, but the parents may not represent the extremes of tryptophan percent.

Fully recognizing the limitations imposed upon the Castle-Wright

formula by the material used in the present study, minimum gene numbers determining protein, zein, tryptophan, valine, leucine, and isoleucine percentages as estimated by this formula are given in Table 30.

Table 30. Estimated minimum gene number determining quantitative characters in Group I

Character	Minimum gene number
Protein %	22
Zein %	6
Tryptophan%	15
Valine %	8*
Leucine %	8
Isoleucine %	6

*Geometric mean of parent variances used instead of F_1 variance as an estimation of environmental variance.

The minimum number of genes determining protein percentage was calculated as 22. Another indication that a small number of gene pairs determines protein percentage in this cross was the fact that in the F_2 (Table 4), which contained only 102 individuals, the P_2 mean protein percentage was recovered and that of the P_1 was almost recovered.

Zein percentage was determined to be controlled by a minimum of six pairs of factors, while tryptophan, valine, leucine, and isoleucine percentages were determined by fifteen, eight, eight, and six factor pairs, respectively. The important point to be made from Table 30 is that the number of genes determining each character is relatively small.

Discussion

The writer is unable to find any literature relating to the inheritance of zein, valine, leucine, or isoleucine, but several studies have been found on the inheritance of total protein and one on tryptophan.

The data reported in this paper indicating dominance of low protein percentage are in agreement with those of East and Jones (18) in corn, of Clark (12), Clark and Hooker (13), and Clark and Quisenberry (14) in wheat, and of Barbacki (2) in barley. In both groups low protein percentage was completely dominant, as shown by the fact that the F_1 and Be_2 means were not significantly different from their respective P_2 means. Similarly, low tryptophan, valine, and isoleucine percentages were completely dominant in the Group I material, but low leucine percentage was only partially dominant.

It should be pointed out that the extreme state of dominance shown by this material may be partially caused by hybrid vigor. In general, nitrogen is the nutrient limiting growth for higher plants. Although the amount of protein produced is limited, this does not hinder production of carbohydrates by photosynthesis. In other words, hybrid vigor shows up in the non-protein fraction of corn more than it does in the protein fraction, thus tending to lower the protein percentage. This same principle applies to protein components as well as total protein.

With respect to the nature of interaction of genes determining

quantitative characters, the writer realises that working with percentage data can be rather misleading at times, but the data in the present study could not be handled in any other manner because the ears were hand-pollinated, resulting in poor seed set in many cases.

In general, the data of Group I fit the arithmetic gene interaction scheme better than that of geometric gene interaction. These data conform very well to the findings of Fraps (20) working with vitamin A content in corn, and of Neal (46) working with yield. Kinman and Sprague (29) also found that yield in corn most closely approximated the values calculated on arithmetic gene interaction. Group II data do not fit either the arithmetic or the geometric schemes.

Arithmetic gene interaction does not necessarily mean that a gene always adds a certain amount to the variable in question, but rather it is a measure of the average amount added over all genic backgrounds.

The number of genes determining protein percentage in Illinois high and Illinois low protein strains of corn is apparently small. The Castle-Wright formula for estimating minimum gene number showed 22 gene pairs determining protein percentage. This is a smaller number than that postulated by "Student", but fits well with the conclusions of Boyce (8), namely, that "Student's" estimate of gene number determining oil percentage in corn is too high. The true situation in this case may well be that protein percentage in corn is determined by a relatively small number of major genes and a larger number of modifier genes. Modifier genes, each having a small effect, are postulated because

it is still possible to raise the mean of the high protein parent slightly by selection. The hypothesis presented here needs to be tested by locating major genes on certain chromosomes through the use of translocations or marker genes before it is accepted.

If the findings of this section prove to be generally true, they could have a rather important bearing on the production of hybrids for specific protein purposes. First, dominance of low protein percentage requires that all of the inbreds going into a hybrid be of high protein percentage. This means that very few of the present inbreds could be used in high protein combinations, and thus a new series of inbreds would have to be isolated or the old ones modified. The same holds for protein components. Second, if the number of major genes determining protein or protein component content is small, it will be a fairly easy process to isolate high protein inbreds,or, by a backcrossing procedure, to modify existing inbreds.

Summary

Individual ear samples of Illinois high and Illinois low protein corns, and of the F_1, F_2, Bc_1, and Bc_2 from a cross between the two were analyzed for protein, zein, tryptophan, valine, leucine, and isoleucine. Protein, zein and tryptophan analyses were made on similar material for the cross Hy x Il98. From these data the following conclusions were drawn:

1. Low protein percentage and low zein percentage were completely dominant in both crosses. Low tryptophan percentage was completely dominant in Group I, but not so in Group II. Valine and isoleucine percentages showed complete dominance on the low side, while low leucine percentage was only partially dominant.

2. It was suggested that the protein differences between high and low protein corns are determined by a few major genes and a large number of minor modifying genes.

3. The nature of the interaction of genes determining protein, zein, tryptophan, valine, leucine, and isoleucine percentages was found to be arithmetic in the high x low protein cross.

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APPENDIX

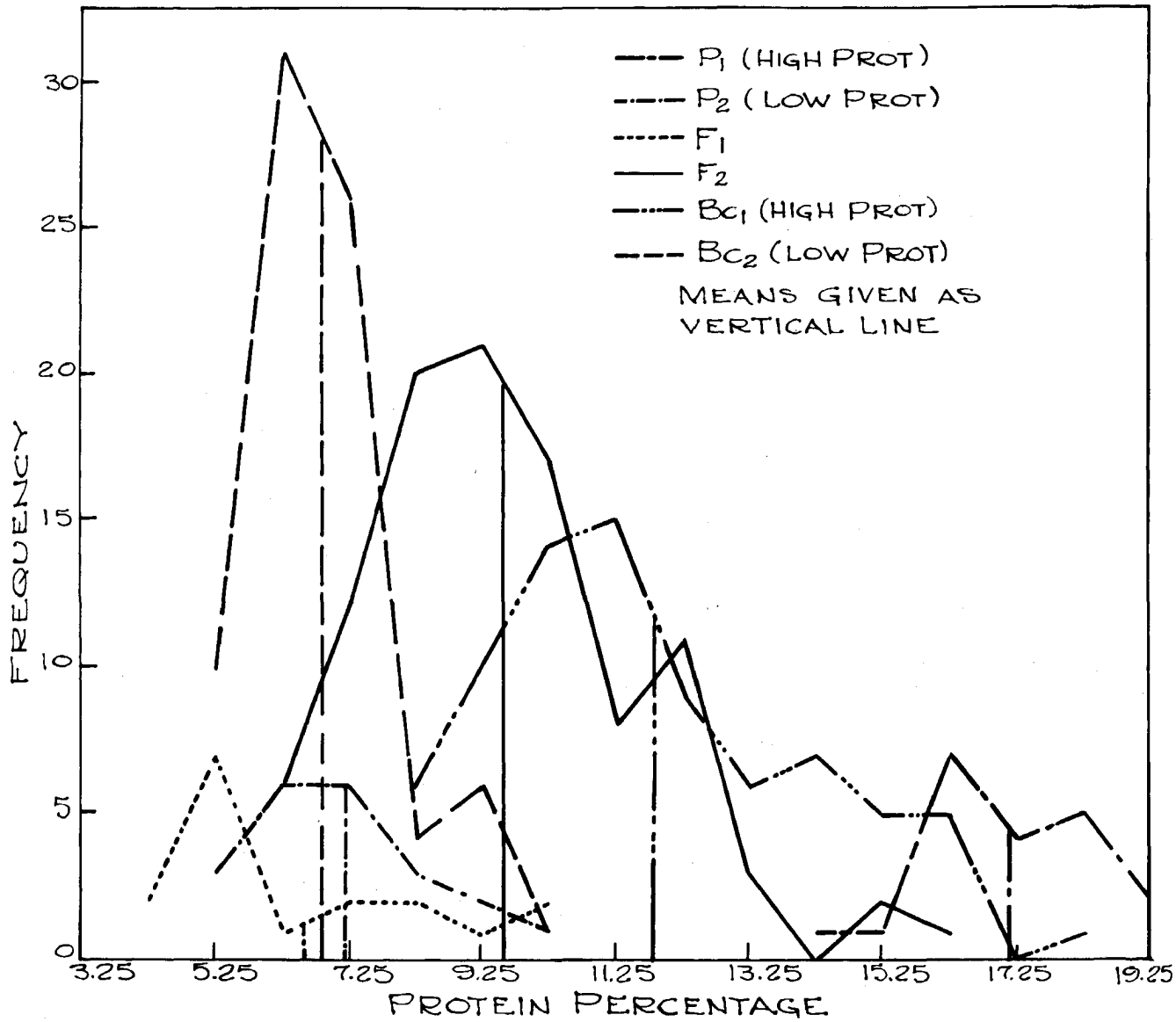

Figure 3. Frequency distributions of protein percentages in Group I material

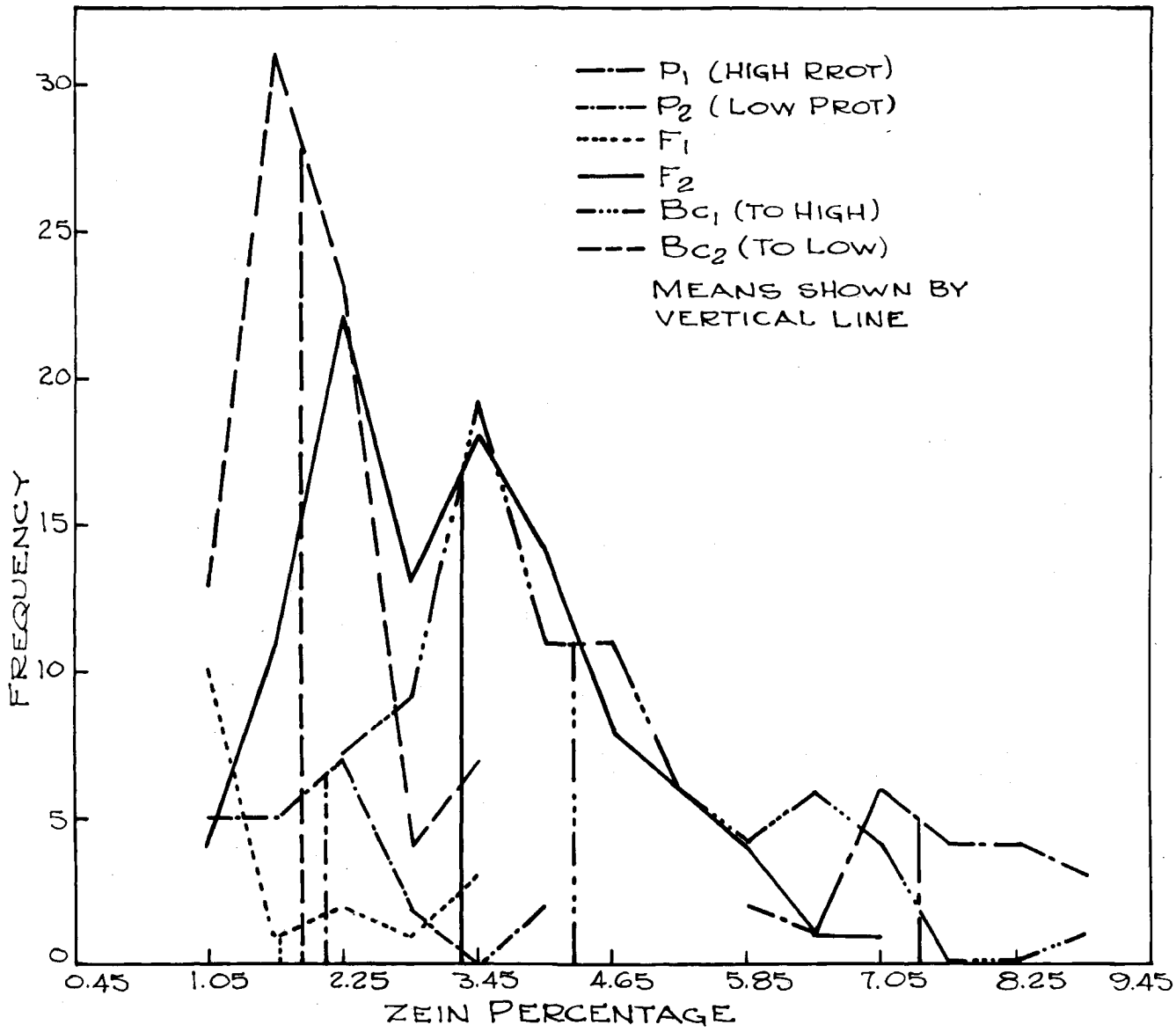

Figure 4. Frequency distributions of zein percentages in Group I material

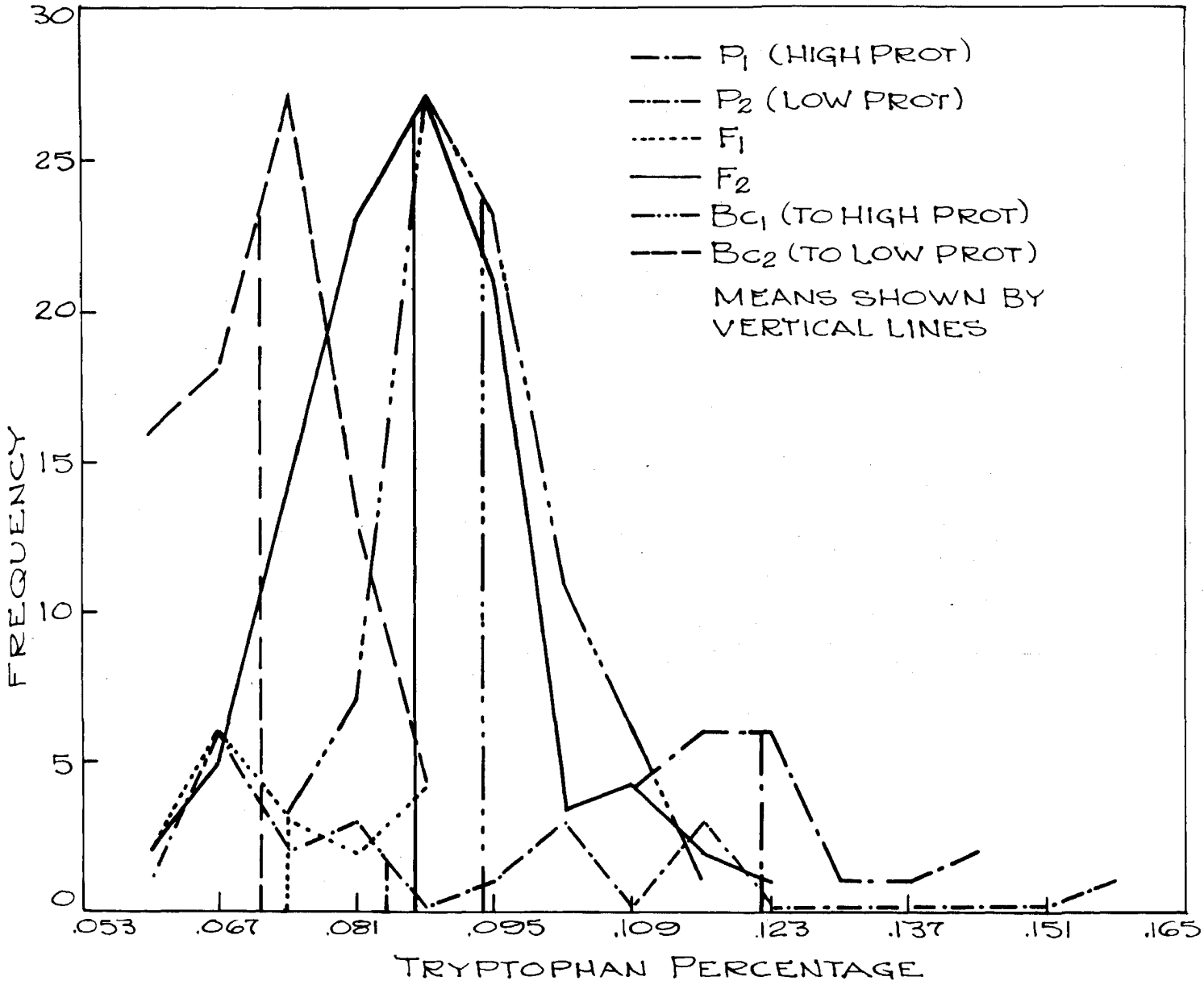

Figure 5. Frequency distributions of tryptophan percentages in Group I material

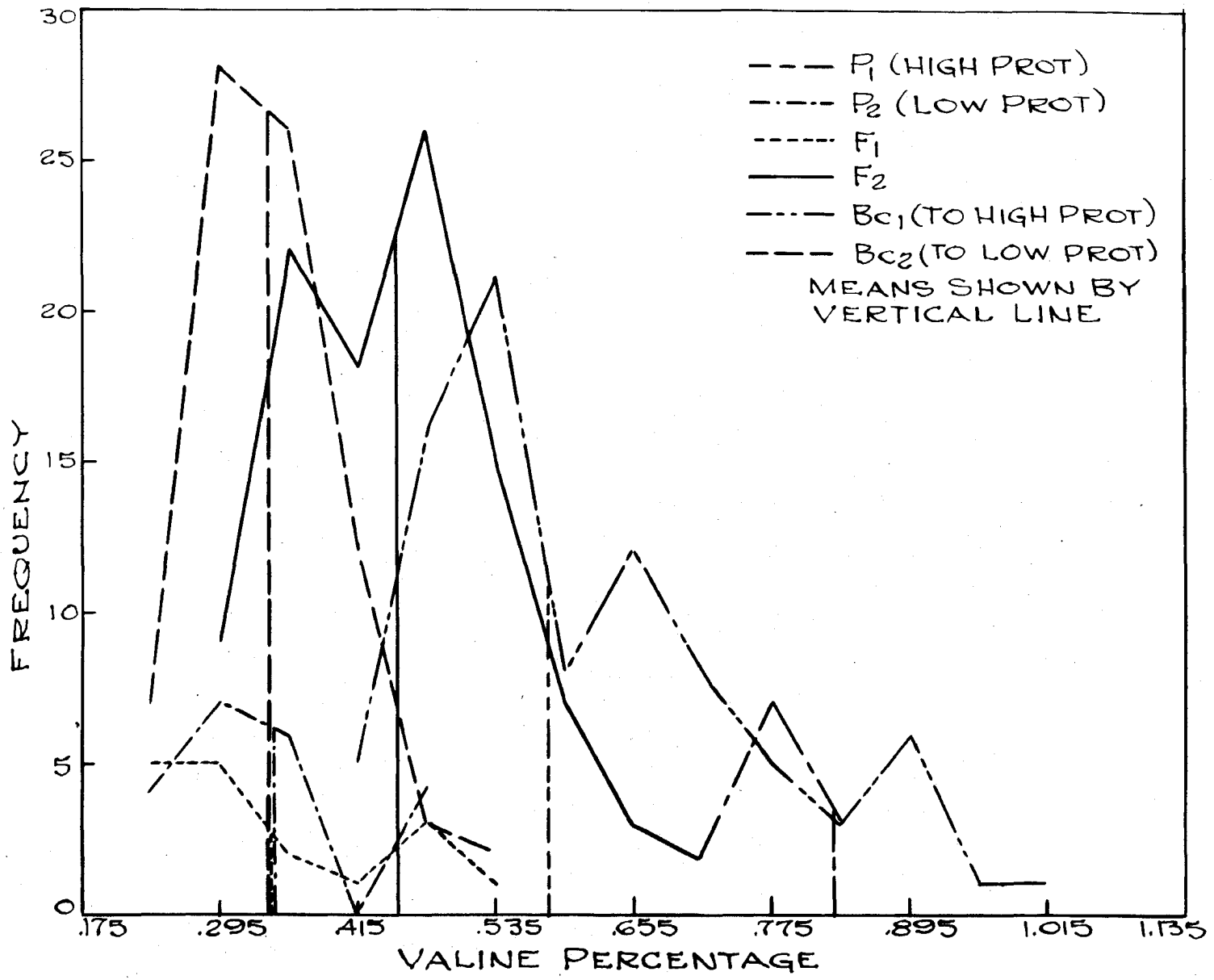

Figure 6. Frequency distributions of valine percentages in Group I material

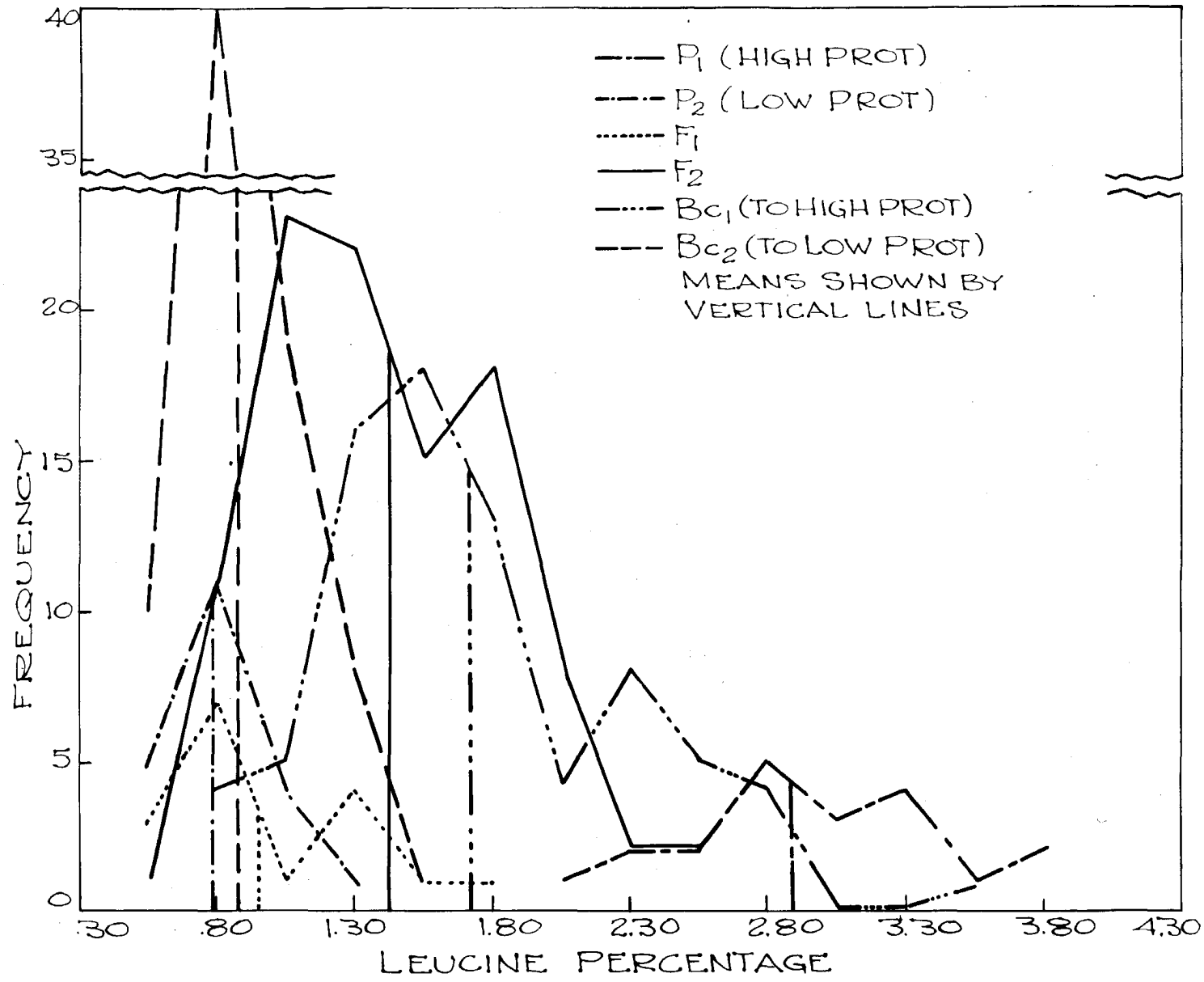

Figure 7. Frequency distributions of leucine percentages in Group I material

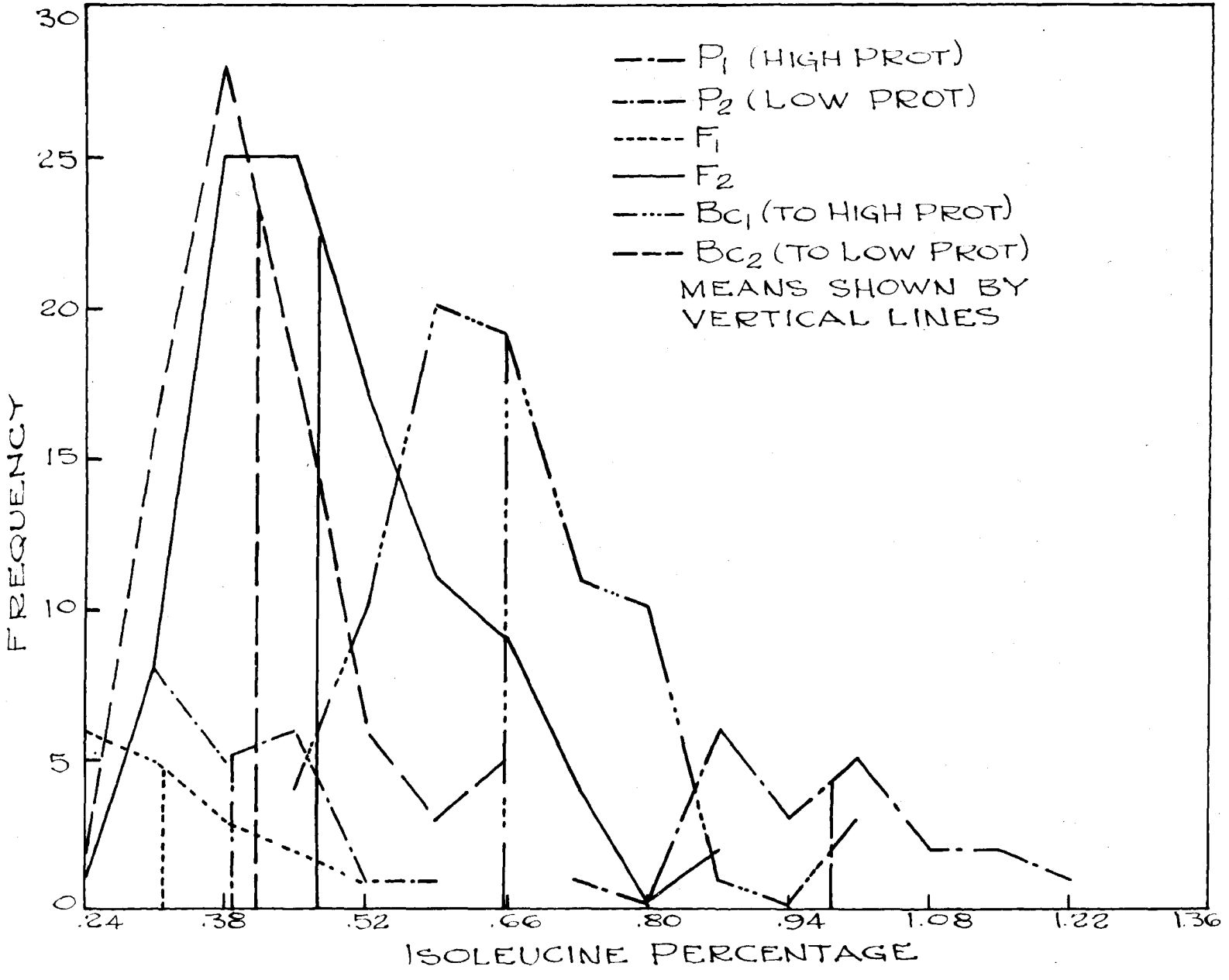

Figure 8. Frequency distributions of isoleucine percentages in Group I material

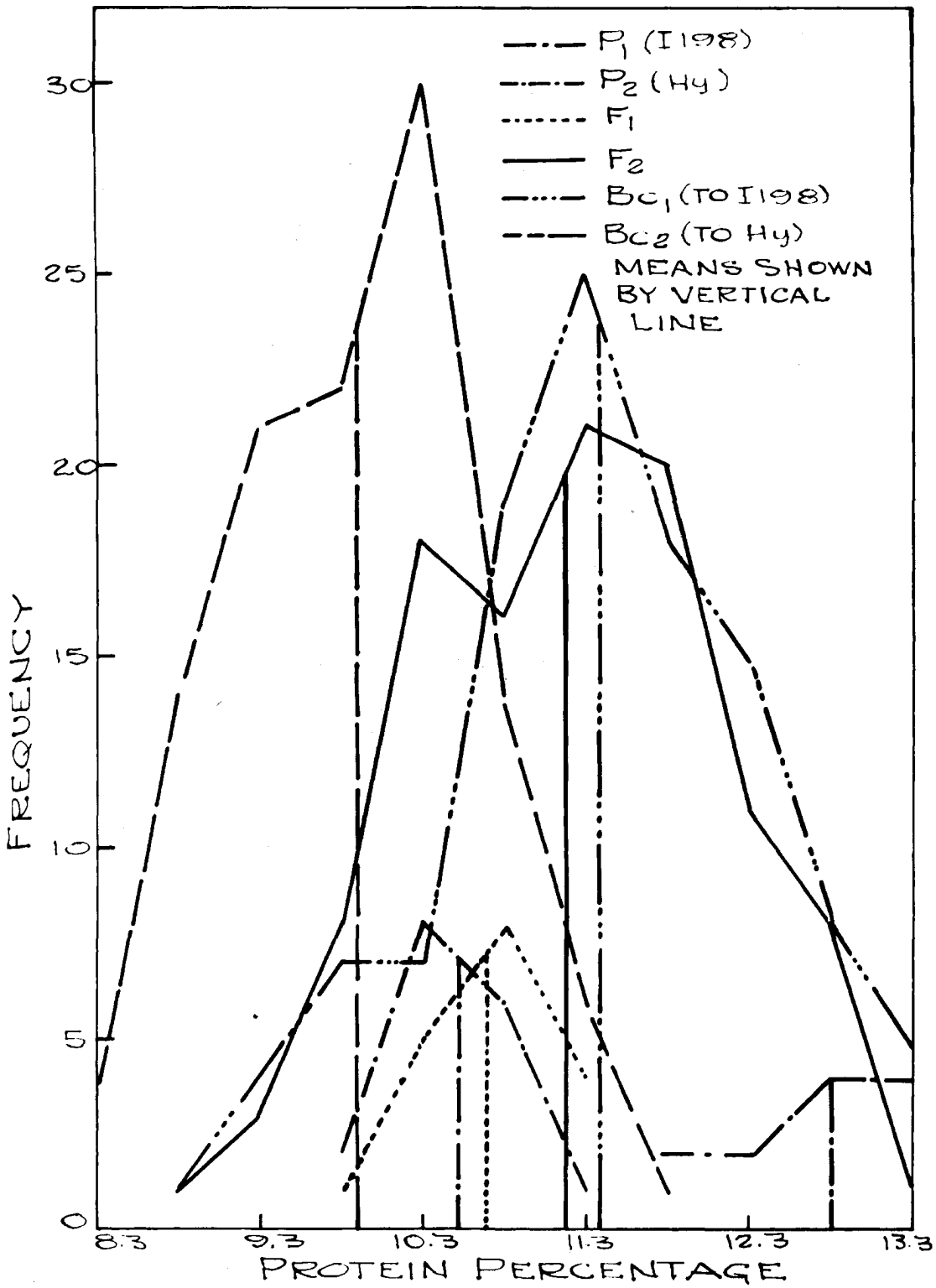

Figure 9. Frequency distributions of protein percentages in Group II material

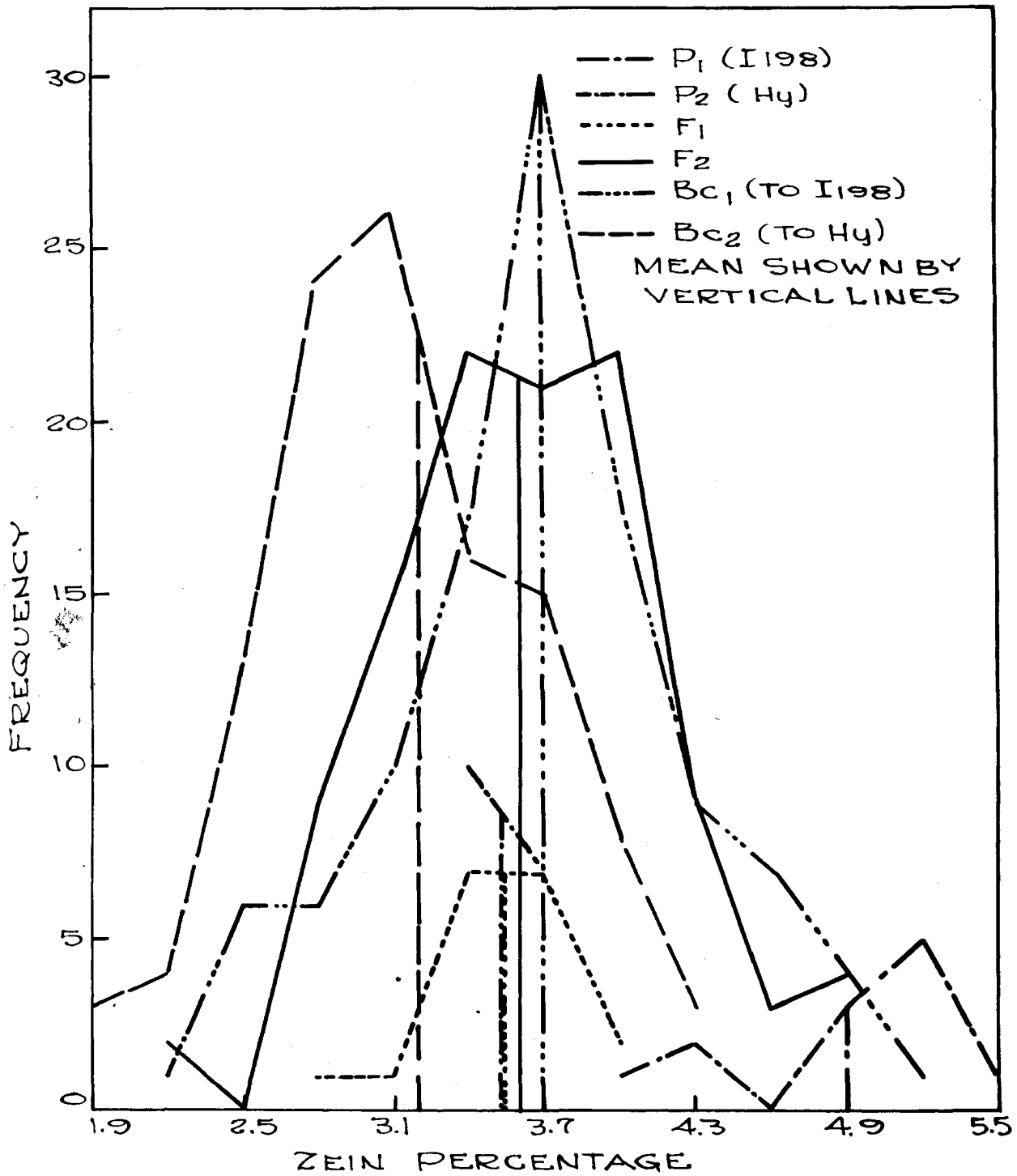

Figure 10. Frequency distributions of zein percentages in Group II material

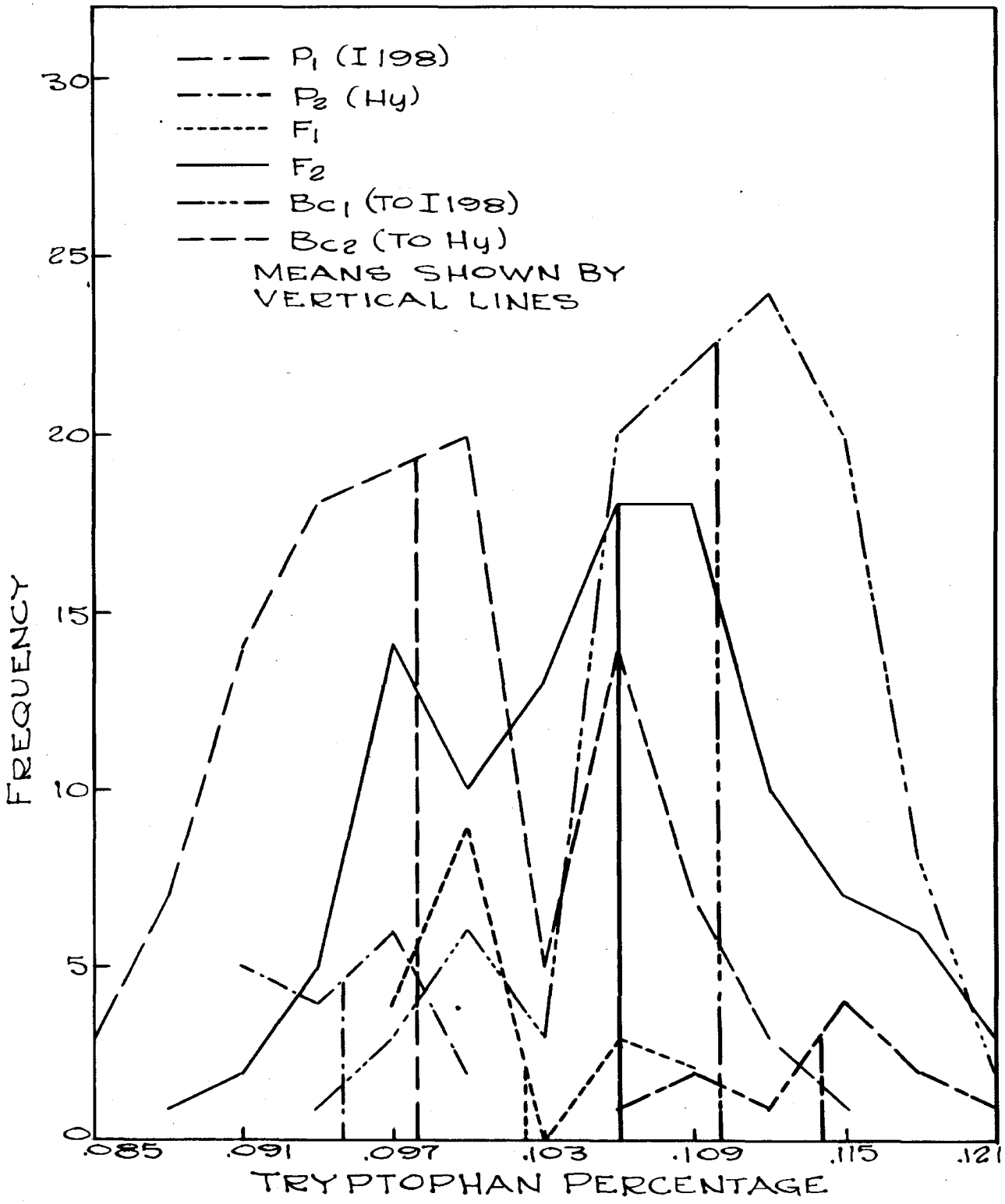

Figure 11. Frequency distribution of tryptophan percentages in Group II material

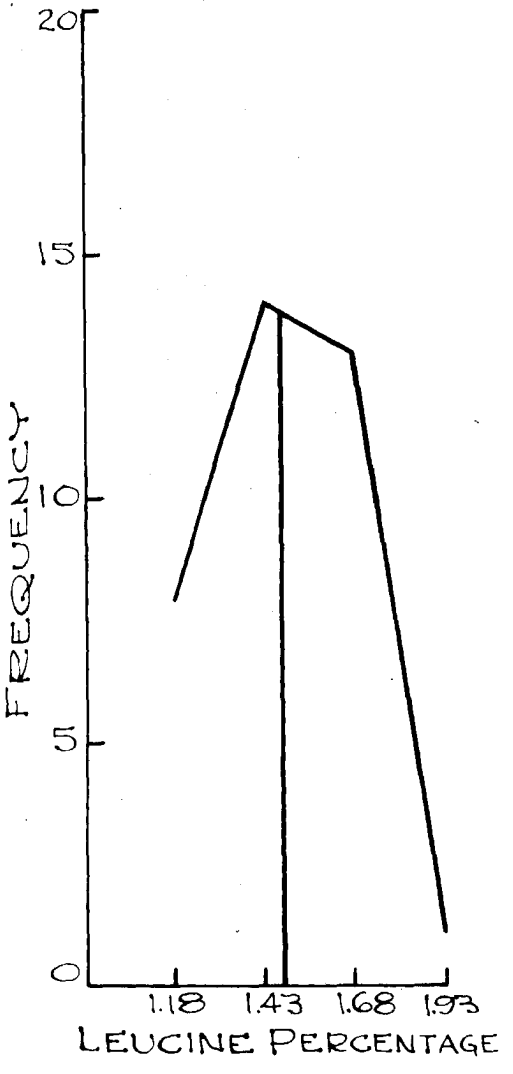

Figure 12. Frequency distribution of leucine percentages in the F_2 of Group II

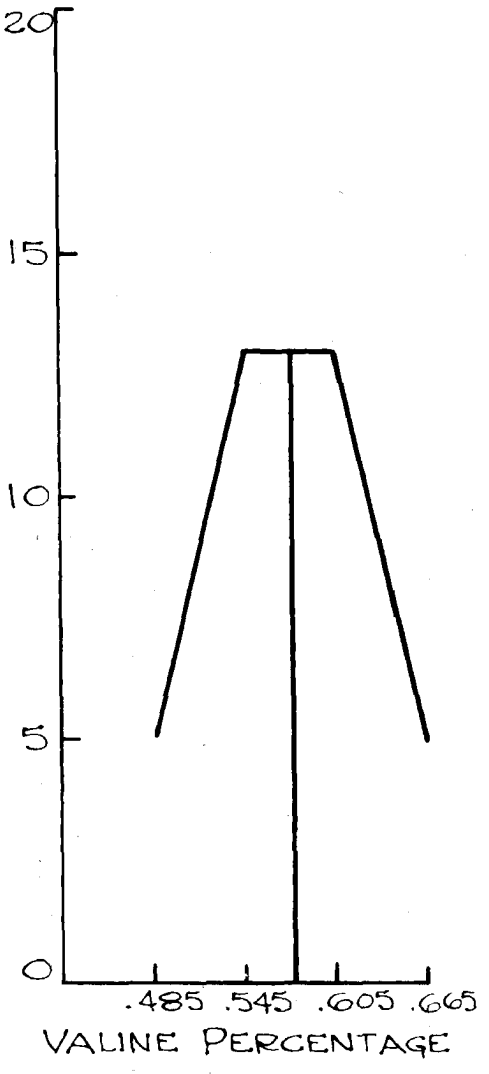

Figure 13. Frequency distribution of valine percentages in the F_2 of Group II

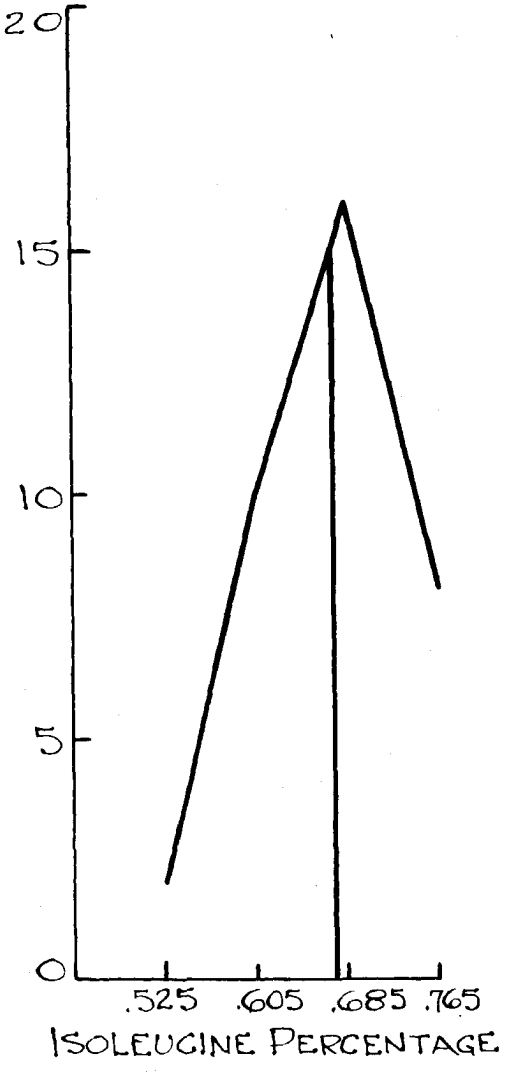

Figure 14. Frequency distribution of isoleucine percentages in the F_2 of Group II