Analyses of Plant Materials Using EDTA Salts as Solubilizers

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The determination of calcium and other metals in plant materials by the usual method of ashing, often twice ashing, is slow and rather involved and destroys organic matter that may be of interest. In a rapid method that avoids ashing a solution of ethylenediaminetetraacetic acid (EDTA) salts successfully extracts all the calcium from certain ground or pulped plant tissues. The resultant extract can then be analyzed by gravimetric, flame spectrophotometric, polaroscopic, and chromatographic methods.

A BASIC LEAD ACETATE SOLUTION is useful for extracting certain constituents from pulped plant materials. This lead water extract can be used for determining the sodium, potassium, and sugar content. In an attempt to develop a test method for calcium in plant materials, experimentation with beet root pulp showed that water or basic lead acetate solution will extract only a small portion of the calcium present in the pulp.

The procedure usually recommended for the determination of minerals in plant materials required the wet or dry ashing of the sample as a necessary step preliminary to the determination of total calcium (*1, 3, 6, 7, 11, 13, 15, 16, 21, 24*). When dry ashed, many samples fuse to such an extent that it is necessary to lixiviate the ash and then reburn the residue, this makes the routine analysis of many samples very cumbersome. Vanstone (*19*) and Vanstone and Philcox (*20*) suggested feeding a dry powder into the flame, even here the sample must be ashed or dried and powdered first. Morgan (*17*), and Dahlberg and Brown (*12*), proposed the use of an acetate solution as an extractant to solubilize the metals to be tested for in soils. This method has been elaborated by Nicholas (*18*) for plant materials. Carolan (*10*) suggested using water or lead acetate solution. The disadvantage of Morgan's acetate solution, or lead acetate solution, or water is that some metals in some plants—e.g., calcium in beet roots—are firmly bound and not entirely solubilized. If strong acids are used to solubilize the metals, interesting organic compounds—e.g., sugars—are destroyed.

The method proposed here utilizes the solubilizing effect of EDTA [ethylenediaminetetraacetic acid, (ethylenedinitrilo)tetraacetic acid] salts to remove the calcium from the plant tissue and provides a solution suitable for the determination of inorganic and organic constituents. Distinction should be made between this method, and previous methods (*15, 22, 23*) that utilize EDTA as a standard titrant for the determination of calcium in solution. The technique proposed here is that of preparing an extract of the plant tissue using a solution of an EDTA salt. The extract contains, solubilized from the pulped plant material, all of the sodium, potassium, calcium, and some other metals. It also contains in a suitable form for further analyses, sugars, amino acids, and the other water-soluble organic compounds. The extract has been used for determination of sucrose using a saccharimeter, of other carbohydrates and amino acids by chromatographic separation, and of the metals using flame spectrophotometric or gravimetric methods.

EDTA salts, basic lead acetate, and the substances extracted from the plant all affect the flame intensities. When many analyses of a few similar materials are to be made, it is most convenient to use standard curves that have been compensated (*2, 5, 10, 14*). In this method ion interference, and viscosity effects, are compensated by including in the solutions used to prepare the standard curves about the same concentrations of all materials as are expected to be found in the unknowns. When a few analyses are to be made of many dissimilar materials, it is most convenient to use the method of Bauserman and Cerney (*4*). This method utilizes an internal standard with the Beckman flame spectrophotometer, which compensates for the effects of organic matter. Very few standard curves are required with this method.

Procedures

Apparatus The ground or pulped plant material can be digested in pint Mason jars, which should be sealed and allowed to stand for 30 minutes with occasional shaking. The analyses can be speeded greatly by blending the contents of the jar for 1 to 3 minutes using an Osterizer which fits directly on the jar. The Osterizer, manufactured by the John Oster Mfg. Co., Racine, Wis., was used in these tests.

Spectrophotometric determinations of the metals were made by means of a Beckman Model DU instrument with the Model 10300 flame attachment, burning natural gas with compressed oxygen.

A Bausch and Lomb quartz wedge saccharimeter was used for sugar determinations.

Reagents Disodium EDTA was obtained from two sources. Lot 1 was obtained from the Hach Chemical Co., Ames, Iowa; lot 16 was obtained from the Alrose Chemical Co., now Geigy Industrial Chemicals, 89 Barclay St., New York 8, N. Y. Diammonium EDTA was prepared by neutralizing EDTA (acid form) (Alrose) with ammonium hydroxide. Dr. Horne's dry lead is a powder form of basic lead acetate, obtained from Baker & Adamson.

Methods In this study many analyses were required on the same sample of ground or pulped plant material. To avoid decomposition or alteration, if such was considered possible, the mixed pulped samples were sealed in polyethylene freezer bags and stored in a freezer chest at −15° to 20° C. until needed. Relatively dry samples were passed through a laboratory grinding mill. The resulting meal was suitable for aqueous digestion. This procedure seems to be applicable to any plant material that can be reduced to a suitable pulp or meal. Extremely oily materials might be an exception.

Spectrophotometer calibration curves were made, using standard solutions containing the element to be tested and the other constituents normally present.

Standard Solutions

	Plant Material	Na, P.P.M.	K, P.P.M.	Mg, P.P.M.	Ca, P.P.M.	EDTA salt, %	PbAc, %	Sugar, %
Sodium	High sugar	0–100	150	100	100	1	0.7	2
	Low sugar	0–200	100	100	100	1	0.7	0
	Ashed	0–200	100	100	100	0	0	0
Potassium	High sugar	100	0–450	100	100	1	0.7	2
	Low sugar	100	0–300	100	100	1	0.7	0
	Ashed	100	0–300	100	100	0	0	0
Calcium	High sugar	100	100	100	0–100	1	0.7	2
	Low sugar	100	100	100	0–200	1	0.7	0
	Ashed	100	100	100	0–200	0	0	0

Calcium Determination

By Ashing, Spectrophotometer Method. Weigh out 13.000 grams of the succulent pulped plant materials, or lesser amounts of dry samples. Transfer to a silica crucible and ash at dull red heat (about 550° C.) for 2 hours. Cool, and take up the ashed sample with concentrated hydrochloric acid. Filter, wash with water, make to 100 ml. and reash the residue and filter paper. Take up the residue with concentrated hydrochloric acid, filter, wash, and repeat the following determination on this portion. As an alternative the filtrates might be combined. Using a dry pipet remove exactly 5 ml. of the filtrate from the ashed sample for analysis by the flame spectrophotometer. Measure the calcium line intensity as per cent transmittance, having previously standardized the instrument. Parts per million divided by 1300 equals per cent calcium in the plant material.

By Ashing, Gravimetric Method. To the remaining 95 ml. of filtrate from the ashed sample, add a piece of litmus paper, and make basic with ammonium hydroxide. Heat on a hot plate just to boiling and filter promptly. Wash the precipitate six times with small portions of hot water. Neutralize the solution with acetic acid, then add a few drops more. Heat just to boiling and then add, slowly, 25 ml. of a 4% solution of ammonium oxalate. Slowly add ammonium hydroxide until the solution is alkaline. Allow the solution to stand for 1 hour before filtering. Filter and wash the precipitate with several small portions of hot water. Burn off the filter paper and heat the precipitate at dull red heat for 2 hours. Cool, moisten with a saturated solution of ammonium carbonate, dry at 110° C., and weigh the precipitate. Remoisten with ammonium carbonate, dry, and reweigh. Repeat to a constant weight. From the weight of the residue as calcium carbonate calculate the per cent calcium in the plant material. Alternatively the AOAC method for the determination of calcium in plant material might be used (*1*).

By Digestion, Using Horne's Dry Lead or EDTA Salt. Weigh out a saccharimeter half normal weight of juicy pulped plant material (13.000 grams), transfer to a pint Mason jar, then add 88.5 ml. of EDTA salt solution and, if sugar determinations are desired, just sufficient Horne's dry lead to clarify (*8*, *9*). Air-dried samples such as grains and straws are digestible in the Osterizer when 1.0 to 5.0 grams of the air-dried and ground material are added to sufficient water to make 100-ml. total volume of solution. Digest the pulp with a blender for 1 to 3 minutes or digest the pulp cold, with frequent shaking for 30 minutes, then filter. Obtain the transmittance value for calcium on the flame spectrophotometer using the appropriate settings. Parts per million of calcium in solution, as obtained from the curve, is calculated to per cent calcium in the plant material.

Sodium and Potassium Determination by Digestion The filtrate prepared as for the calcium determination is suitable for the determination of sodium, and of potassium, provided the diammonium salt of EDTA is used in place of the disodium salt.

Sugar Determination by Digestion The filtrate prepared as for the calcium determination is suitable for the determination of sugar with the saccharimeter, if only just sufficient Dr. Horne's dry lead is added to clarify the filtrate, as described by Browne and Zerban (*8*).

Experimental

Certain plant materials—e.g., those apple fruits and cabbage leaves tested—required little EDTA treatment. Other plant materials, such as rhubarb petioles, required considerable quantities of EDTA salt to solubilize the calcium in the pulp. Experiments using disodium EDTA indicated that this particular lot of material contained considerable quantities of calcium. A solution of 1 gram of disodium EDTA, 2 grams of sucrose, and the usual quantities of other normal constituents was made to 100 ml. and was analyzed in the spectrophotometer with its appropriate blank. The 1 gram of disodium EDTA increased the calcium by 9 p.p.m. in the solution. More of the same lot of disodium EDTA was ashed and then analyzed. Results in this case showed 0.09% calcium in the EDTA salt, the same amount as found by the other method. Other lots of EDTA salts contained larger or smaller quantities of calcium. The EDTA material should be tested for contaminants and the analyses corrected accordingly.

The experiment in Table I was performed to determine the forms of EDTA suitable for solubilizing the calcium in beet pulp. Either the disodium or diammonium salt is satisfactory, but the acid form is not, probably because of its low solubility.

In order to avoid adding an undue excess of EDTA salt, the experiment reported in Table II was performed. As 0.1 gram of EDTA salt is sufficient to solubilize the calcium from samples containing about 0.020%, it was decided to use an excess to the extent of 1.0 gram of EDTA salt per sample. This excess has been sufficient for all the thousands of beet root samples tested in this laboratory. The amount of EDTA salt required will vary for different plant materials. Table III shows the experimental values obtained when various quantities of disodium EDTA were added to replicated portions of pulped apple fruits, carrot roots, potato tubers, cabbage leaves, and rhubarb petioles. For the apple samples 1.0 gram of EDTA salt was sufficient. Other tests indicate that all of the calcium may be solubilized from some apple fruit, using even less EDTA salt. For potato tubers, sugar beet roots, carrot roots, and cabbage leaves, 1.0 gram seems to be suitable. For rhubarb petioles, 3.0 grams of the EDTA salt in the extracting solution has been sufficient.

A simple method for determinations on single samples of unfamiliar material can be developed, as shown in Table III. If increasing quantities of EDTA salt do not solubilize increasing amounts of calcium, it can apparently be safely inferred that all the calcium has been solubilized.

The volume of extraction water or

Table I. Calcium Concentration in Extract and in Portions of Ashed Pulp
(Beet pulp treated with 1.00 gram of EDTA and lead acetate. Determinations by spectrophotometer)

Sample No.	Calcium, %						
	$(NH_4)_2EDTA$	NA_2EDTA	EDTA acid form	$(NH_4)_2EDTA$ + lead	NA_2EDTA + lead	EDTA acid form + lead	Ashed plant material
5003	0.017	0.017	0.008	0.017	0.016	0.008	0.019
5037	0.022	0.023	0.008	0.024	0.023	0.008	0.021
5038	0.025	0.025	0.008	0.025	0.025	0.008	0.023

Table II. Effect of Treatment on Calcium Content of Beet Pulp Extract

Sample No.	Calcium, %: Grav. detn. on ashed pulp	Determination by Spectrophotometer: On ashed pulp	Cold Digestion, 88.5 Ml. Water: 1 gram Na_2EDTA	0.1 gram Na_2EDTA	Water only	Horne's dry lead only
1	0.021	0.022	...	0.020	0.011	0.008
2	0.028	0.028	...	0.020	0.012	0.007
3	0.019	0.021	...	0.017	0.010	0.008
4	0.029	0.026	...	0.020	0.008	0.008
5	0.016	0.021	...	0.017	0.009	0.006
6	0.029	0.024	...	0.023	0.012	0.008
7	0.020	0.018	0.021	0.016	0.007	0.007
8	0.018	0.018	0.020	0.016	0.007	0.007
9	0.024	0.022	0.023	0.020	0.008	0.006
10	0.019	0.018	0.024	0.021	0.009	0.007
11	0.020	0.020	0.019	0.023	0.009	0.008

Table III. Calcium in Ash of Fruits and Vegetables

Plant Material	T[a], %	Ca, P.P.M.: Uncorr.	EDTA	Corr.	Ca, %
		1 Gram Na_2EDTA (Lot 16)			
Apple	41.8	30	17	13	0.010
Carrot	59.0	55	17	38	0.029
Potato	52.0	45	17	28	0.022
Cabbage	70.2	72	17	55	0.042
Rhubarb	55.8	51	17	34	0.026
		2 Grams Na_2EDTA (Lot 16)			
Apple	...	..	..	..	...
Carrot	67.8	68	34	34	0.026
Potato	65.5	65	34	31	0.024
Cabbage	81.0	88	34	54	0.042
Rhubarb	74.8(1:1)	158	34	124	0.095
		3 Grams Na_2EDTA (Lot 16)			
Apple	...	..	..	..	...
Carrot	78.0	83	51	32	0.025
Potato	74.2	78	51	27	0.021
Cabbage	91.8	103	51	52	0.040
Rhubarb	87.8(1:1)	196	51	145	0.112
		Ashed Pulp			
Apple	9.1	12	..	12	0.009
Carrot	22.6	43	..	43	0.033
Potato	14.0	22	..	22	0.017
Cabbage	27.0	54	..	54	0.042
Rhubarb	65.8	146	..	146	0.112

[a] Transmittance dial reading which is spectral line intensity.

solution added was determined by the method of Sachs–Le Docte (*9*), and is chosen so that the liquid in the plant plus the extracting solution will total nearly 100 ml. Previous work has shown that if 13 grams of beet pulp are used, 88.5 ml. of water will give a total solution volume of nearly 100 ml.; 88.5 ml. of extracting solution was used for all the succulent plant material in these tests. This is not strictly correct, as allowance should be made for the fact that the water content is variable in dissimilar plant material.

To test the method on relatively dry plant material, oat straw, wheat grain, and milo grain were ground to pass a 20-mesh screen. One to 5 grams of these samples were used, dependent upon anticipated calcium content. Sufficient EDTA solution was used to make 100 ml. of total solution, 100 minus the per cent moisture content of the sample will give approximately the correct amount of EDTA solution to add. Table IV shows that diammonium EDTA solution will extract the calcium from samples of dried plant materials.

In certain instances it is advantageous to use a single extract for more than one determination. In case sodium (and also potassium) determinations are required, the diammonium salt of EDTA can be substituted for the disodium salt. The diammonium EDTA and EDTA acid form, used to prepare the diammonium salt, were generally much more pure than the disodium salt and therefore needed little or no correction for blank. The disodium and diammonium EDTA salts appear to sequester the calcium equally well. Many tests were run; three results are shown in Table I and two examples in Table V. Table V also compares per cent sucrose as determined by the usual lead acetate method of clarification with the determination by the EDTA method.

The method calls for the addition of a solution of the EDTA salt. If basic lead acetate is used to clarify the filtrate, the order of addition of the reagents is of some consequence. If sufficient lead salt, but not a great excess, is used to clarify the filtrate, the final equilibrium is that of complete solution of the calcium. If the lead salt is added first, a certain coagulating action seems to seal the particles of plant material, so that a relatively long time is required to solubilize the calcium. If, on the other hand, the EDTA salt can effect the solubilizing before the lead salt has acted, the digest mixture can be filtered soon. Table VI shows this effect. Diammonium EDTA was used in this series.

In partial summary Table VII gives the results obtained on replicated portions of the same samples. The results would seem to indicate that the differences in determinations of all three elements can be attributed to random variables in sampling and analysis. The per cent error in potassium determinations is no greater than that for the other elements.

Discussion and Conclusions

Prolonged mixing of pulped plant material is necessary before duplicated determinations will yield consistent results. During much mixing, most plant materials suffer various changes. The sucrose is degraded and enzyme systems affect certain amino acids. If such sensitive materials are of interest, a compromise between mixing and accuracy must be reached. A solution of the disodium and diammonium salts of EDTA solubilizes all of the calcium in all the plant materials tested, and probably in most biological materials. EDTA (acid form) is not suitable. This work indicates that the quantity of EDTA salt required may be judged by adding increasing increments of EDTA to the sample. If additional amounts of EDTA do not yield additional calcium from the plant in the extract, all calcium has probably been completely solubilized. It is possible to choose such a soluble EDTA salt that other metals can be

Table IV. Calcium in Ash of Dry Grain

(Determination by spectrophotometer and EDTA digestion)

Sample	Calcium in Air-Dry Sample, %: Osterizing $(NH_4)_2$ EDTA + lead	Ashed
Oat straw	0.168	0.160
	0.160	0.160
Wheat grain	0.048	0.052
	0.048	0.052
Milo grain	0.032	0.036
	0.032	0.036

Table V. Typical Analyses of Beet Pulp Extract

Beet No.	Calcium, %			Sucrose, %			Sodium, %		
	Pb water extract	$(NH_4)_2EDTA$ only	Na_2EDTA only	Pb water extract	$(NH_4)_2EDTA$ + Pb	Na_2EDTA only	Pb water extract	$(NH_4)_2EDTA$ + Pb	Na_2EDTA only
1	0.007	0.038	0.036	10.1	10.1	Turbid	0.186	0.184	...
2	0.007	0.033	0.036	13.0	13.0	Turbid	0.096	0.092	...
3	...	0.025	...	18.3	18.2	...	0.003	0.003	...
4	...	0.024	...	17.4	17.9	...	0.005	0.005	...
5	...	0.024	...	16.5	16.9	...	0.005	0.005	...
6	...	0.027	...	17.1	17.3	...	0.006	0.005	...
7	...	0.028	...	17.4	17.8	...	0.002	0.003	...
8	...	0.018	...	16.5	17.0	...	0.012	0.011	...

Table VI. Effect of Sequence of Addition of Materials to Extracting Solutions

Test No.	Order of Addition and Treatment		Time after Blending 1 to 3 Minutes					
	First blending after adding	Second blending after adding	Immediately		2 Hours		24 Hours	
			T, %	Ca, %	T, %	Ca, %	T, %	Ca, %
1	EDTA solution only	....	46.3	0.031	47.0	0.032	..	...
2	Dry EDTA + water	.....	46.0	0.031	46.5	0.032	..	...
3	Dry lead + dry EDTA + water	....	23.8	0.008	30.0	0.015	46.8	0.032
4	Dry lead + water	Dry EDTA	26.5	0.012	46.8	0.032	..	...
5	Dry EDTA + water	Dry lead	47.0	0.032	47.1	0.032	..	...
6	EDTA soln. + dry lead	....	46.2	0.031	47.1	0.032	..	...

Table VII. Analyses of Solutions of Pulped Plant Extracts Prepared by Three Methods

Pulped Plant Material	Calcium, %			Sodium, %			Potassium, %		
	Osterizing $(NH_4)_2EDTA$ + Pb	Ashing	Osterizing Pb	Osterizing $(NH_4)_2EDTA$ + Pb	Ashing	Osterizing Pb	Osterizing $(NH_4)_2EDTA$ + Pb	Ashing	Osterizing Pb
Beet									
1	0.032	0.029	0.015	...	0.036	...	...	...	...
	0.032	0.029	0.015	...	0.036	...	...	...	...
	0.032	0.029	0.010	0.032	0.034	0.038	0.162	0.186	0.191
	0.032	0.031	0.010	0.032	0.034	0.038	0.162	0.186	0.187
2	0.030	0.029	0.008	...	0.128	...	...	...	...
	0.029	0.031	0.008	...	0.128	...	...	...	...
	0.030	0.031	0.005	0.120	0.123	0.123	0.158	0.187	0.187
	0.029	0.031	0.005	0.120	0.123	0.123	0.158	0.187	0.191
Rhubarb 3	0.015	0.012	0.003	0.007	0.005	0.006	...	...	...
	0.015	0.012	0.003	0.007	0.007	0.006	...	...	...
	...	0.012	...	...	0.007	...	...	0.157	...
Cabbage 4	0.028	0.023	0.025	0.025	...	0.025	...	...	...
	0.028	0.023	0.025	0.025	...	0.025	...	...	...
	0.031	0.027	0.031	0.027	0.028	0.027	0.263	0.254	0.258
	0.029	0.028	0.031	0.027	0.028	0.027	0.257	0.254	0.254
Apple 2	0.006	0.006	0.006	0.006	0.005	0.007	...	...	...
	0.006	0.006	0.006	0.006	0.003	0.007	...	...	...
	0.008	0.008	0.008	0.005	0.003	0.004	0.115	0.137	0.138
	0.008	0.008	0.008	0.005	0.003	0.004	0.112	0.136	0.135

determined on the same plant extract. The extract can be analyzed by spectrophotometric or gravimetric means. An experiment planned for future study is the possibility of determining calcium by titration of the excess EDTA present in an aliquot of the extract.

Basic lead acetate can be used to clarify the extract during or after digestion. Such treatment facilitates the later determination of certain carbohydrates and amino acids in the extract. Care should be exercised in the use of lead salts, however, to avoid affecting the solubilizing of the calcium.

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CROP HEREDITY AND NUTRITIONAL QUALITY

Inheritance and Heritability of Protein, Niacin, and Riboflavin in Oats

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In three segregating populations of oats it was found that the genes determining high protein percentage could be either recessive or dominant, depending upon the genetic background on which they operated. With one parent, the high protein percentage behaved as a dominant in one cross but recessive in the other; for another parent, low protein percentage behaved as the dominant in one cross but recessive in another. High niacin and riboflavin content in oats appeared to be dominant in each of the oat crosses. Heritability percentages for niacin and protein content ranged from 83 to 93, while those for riboflavin ranged from 0 to 52.

LARGELY AS THE RESULT of research work in animal nutrition and plant breeding, there has recently developed an interest in the development of crop varieties which have improved nutritional qualities. The plant materials receiving the greatest attention with respect to nutritional quality are the grains of the cereal crops and the vegetative tissues of the forage crops. Prior to the effective selection of crop varieties with improved nutritional qualities, it will be necessary to establish the mode of inheritance of chemical components of the crops being investigated.

The chemical components of the cereal grains which have received the most attention are protein, several amino acids, and four of the B vitamins. Protein and oil content of corn was shown to be an inherited characteristic in the classic experiments on protein and oil selection conducted at Illinois (*6*). Several other investigations have shown that protein content in cereal grains is usually multigenically inherited with low protein percentage dominant (*3, 4*).

Burkholder, McVeigh, and Mayer (*1*), Ditzler, Hunt, and Bethke (*2*), and Richey and Dawson (*10*) found that the variability in the niacin content of corn grain was determined largely by heredity, a large number of genes being involved in the determination of this character. Richey and Dawson suggest that the genetic constitution of the corn endosperm determines in large part the niacin content of the grain, and that the genes for high niacin content are recessive. Frey and Watson (*5*) found large differences among oat varieties with respect to niacin and riboflavin content, with some strains containing twice the content of others. Frey and others (*4*), reporting on the inheritance of niacin, riboflavin, and protein in two oat crosses, found transgressive segregation for the first two components. The mean heritability values for niacin and riboflavin content of oat grain were 50 and 49%, respectively, while that for protein was only 15%.

The purpose of this paper is to give additional information on the inheritance and heritability of protein, niacin, and riboflavin in three oat crosses. It is a sequel to the earlier paper by Frey and others (*4*).

Materials and Methods

The grain from the oat varieties, Mindo, Colo, and C. I. 5298, and of randomly chosen F_3 progenies from the crosses Mindo × Colo, Colo × C. I. 5298, and C. I. 5298 × C. I. 3656, was grown at East Lansing, Mich., in 1952 and subsequently analyzed for niacin, riboflavin, and protein. (Seed of C. I. 3656 was not grown in the same experiment with the other varieties and selections, so the niacin, riboflavin, and protein contents given herein for this strain were collected in 1947, 1949, and 1950 and corrected according to the average of the other parent varieties to a 1952 basis.)

For this study, a number of single plants were randomly selected from the parent varieties and the F_2 population of the three crosses. As the amount of seed from single plants was not sufficient for the chemical determinations, it was necessary to grow F_3 progeny rows from which seed was harvested for analyses. Thus, the chemical contents were determined on the progenies from F_2 selections and supposedly should represent the average genotypes of the F_2 plants. The grain from each selection was prepared for chemical analysis by removing the empty hulls and grinding the sample through a 40-mesh screen.

Niacin content was determined by the method of Krehl, Strong, and Elvehjem (*7*) and the riboflavin content by the method of Snell and Strong (*9*). The nitrogen content of the oat grain was determined by the Kjeldahl procedure and protein percentage was derived by multiplying the nitrogen content by 6.25. The moisture content for the samples was rather uniform, so the analytical data were expressed on an air-dry basis, niacin and riboflavin as micrograms per gram, and protein as percentage of the total.

Experimental Results

Inheritance The frequency distributions for the protein percentages of the selections from the three