pounds in water, the studies were performed in 60% methanol. The results are in general accord with the known specificity of the aminopeptidase.

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Sequential Assays of 4 Amino Acids in Grains of Wheat, Rye, and of Their Hybrid.* (22525)

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The chemical characterization of organisms in ways that may be related to their biological evolution may be taken as a primary objective of comparative biochemistry. A review of peptide analysis(1) listed characterization of proteins for biological differentiation as a principal discernible goal, along with elucidation of amino acid residue sequence. Qualities of an ideal N-terminal agent were also prescribed. The agent with many of these attributes has proved to be fluorodinitrobenzene (FDNB), which has been successfully applied to problems of structure(2). FDNB has received somewhat less use in characterization(3). It has become apparent that a more powerful mode of characterization might be obtained from a method employing quantitative sequential analysis. It therefore proved to be desirable to employ phenylisothiocyanate(4) subtractively(5). Many data have been accumulated by this procedure. A hybrid of wheat and rye(6) afforded an opportunity to investigate chemical characterization as related to biological type. For this purpose, subtractive quantitative sequential peptide analysis was applied to seeds of wheat-rye hybrid (*Triticale*) and to its parents.

For the grains of corn, wheat, and rye, most amino acids have been found not to differ significantly in total content or in proportion in N-terminal and N-penultimate positions (7). As wheat and rye seeds, however, were significantly different in their quantitative contents of total and N-terminal lysine, lysine was chosen for simultaneous assay in wheat, rye, and the hybrid. Assays of leucine were also performed as a basis of comparison inasmuch as the differences in leucine content appeared to be significant. It was furthermore of interest to check on the C-terminality of leucine. Inasmuch as lysozyme is known to contain N-lysine(8,5) and C-leucine(9,10), and the cereals contain N-lysine(7), such analyses permit comparison of whole cereal protein with lysozyme. Methionine and phenylalanine were also studied.

Materials and methods. The parent strains were *Triticum aestivum* and *Secale cereale.* The hybrid, *Triticale,* was generously sup-

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TABLE I. Total, N-Terminal, N-Penultimate, and C-Terminal Contents of 4 Amino Acids in Wheat, Rye, and in Their Hybrid.

N content (%) (MicroKjeldahl)	Wheat (2.45) AT_0	AT_1	AT_2	CT_1	Rye (1.82) AT_0	AT_1	AT_2	CT_1	Lysozyme standard
Leucine N/Total N (%)	*4.3 ± .2*	*4.1 ± .3*	*4.3 ± .1*		*3.9 ± .2*	*3.9 ± .2*	*3.8 ± .3*		6.9 ± .2
	4.3 ± .1	4.3 ± .1			4.0 ± .1	4.1 ± .1			6.5 ± .2
					4.1 ± .1				7.0 ± .2
			4.2 ± .2				3.8 ± .2		7.1 ± .2
				4.1 ± .1				*3.7 ± .1*	6.7 ± .1
				4.1 ± .1				*3.8 ± .1*	7.0 ± .1
Lysine N/Total N (%)	*3.0 ± .1*	*.79± .08*	*1.11± .13*		*1.4 ± .2*	*1.2 ± .1*	*1.46± .08*		5.6 ± .2
	2.9 ± .2			*2.8 ± .2*				*4.0 ± .2*	5.9 ± .2
			1.11± .09				1.82± .22		5.4 ± .2
	2.9 ± .2	1.0 ± .1			4.2 ± .1	1.3 ± .1			5.7 ± .3
Methionine N/Total N (%)	*.69± .02*	*.70± .02*	*.69± .02*		*.72± .02*	*.72± .02*	*.70± .03*		1.77± .06
	.87± .03	.78± .03			.70± .01	.81± .06			1.9 ± .06
	.88± .02				.84± .02				1.87± .07
			.78± .04				.70± .04		1.86± .08
Phenylalanine N/Total N (%)	2.3 ± .1	2.5 ± .05			2.3 ± .1	2.2 ± .1			3.2 ± .1
			2.5 ± .1				2.2 ± .1		3.3 ± .1

N content (%) (MicroKjeldahl)	Wheat × rye Triticale 1.96 AT_0	AT_1	AT_2	CT_1	Lysozyme standard
Leucine N/Total N (%)	*4.6 ± .2*	*4.5 ± .2*	*4.5 ± .2*		6.9 ± .2
	4.3 ± .1	*4.4 ± .1*	*4.3 ± .2*	*4.1 ± .1*	6.7 ± .1
	4.5 ± .2			*4.2 ± .1*	7.0 ± .1
Lysine N/Total N (%)	*3.5 ± .1*	*1.04 ± .07*	*1.26 ± .11*		5.6 ± .2
	3.3 ± .2		*1.05 ± .11*	*3.4 ± .1*	5.9 ± .2
Methionine N/Total N (%)	*.72 ± .02*	*.70 ± .03*	*.69 ± .04*	*.73 ± .02*	1.77 ± .06
Phenylalanine N/Total N (%)	2.5 ± .1	2.3 ± .1	2.3 ± .1	2.4 ± .1	3.1 ± .1

AT_n = Aminoid treatment followed by complete hydrolysis. CT_1 = One carboxoid treatment followed by complete hydrolysis.

plied by Dr. J. G. O'Mara of the Genetics Department. The seeds were ground in a Wiley mill to pass a No. 40 mesh sieve. Moisture contents of the wheat-rye hybrid and its wheat and rye parents were 8.4, 9.1, and 8.7% respectively.

The italicized figures (Table I) comprise values obtained from the hybrid and from its parents. Results on any one line were obtained in a simultaneous set of assays. The non-italicized values represent a non-parental strain of wheat (Rival) and a non-parental strain of rye (spring rye).

The methods have been mostly described (5). Hydrolyses were conducted in excess 3 N hydrochloric acid in an autoclave at 15 lb pressure for 16 hr. No significant differences in results were found when redistilled hydrochloric acid was used under these conditions. Each hydrolyzate was filtered at pH 4 as recommended by Horn, Blum, Gersdorff, and Warren(11).

Assays were controlled by simultaneous assay of the same amino acid in an hydrolyzate of Armour crystalline lysozyme (Lot No. 003L1) with aliquots of the same organism and same medium. The standard deviations (Table I) are calculated from the estimations performed in quadruplicate at each of 5 levels.

The critical subtractive lysine assay method has been shown to be applicable to an N-lysine protein [lysozyme(5)] and to peptides with interior lysine [ACTH(12), bacitracin hydrolyzates(13)]. The use of such methods on unfractionated proteins is further justified by recent results in studies of chymotryptic proteolysis of lysozyme. The present type of analysis on unfractionated mixtures of peptides from proteolysis indicates that 2 of 3 phenylalanine residues in lysozyme are opened at their carbonyl end and that 2 of 3 tyrosine residues in the lysozyme molecule are opened at their carbonyl end by chymotrypsin(14). In a study of aromatic peptides isolated individually from the action of chymotrypsin on lysozyme, Acher, Laurila and Fromageot(15), employing several techniques of structural assignment on isolated peptides, also found 2 of 3 phenylalanine C-residues and 2 of 3 tyrosine C-residues opened by chymotrypsin. Aminoid residues opened by chymotrypsin, as reported in both types of study, comprise those of arginine, aspartic acid (asparagine), glycine, and isoleucine.

Results. The results are presented in Table I. No significant difference is found between each of the 2 strains of either wheat or rye. Significant amounts of free or N-terminal amino acids of those reported cannot be present except in the case of lysine, inasmuch as the other 3 amino acids show after one aminoid treatment a decrement which is less than experimental variation. Lysine also cannot be present in the free state inasmuch as the value following C-terminal treatment is the same as that for the untreated material.

Discussion. Wheat, rye, and wheat-rye hybrid all contain N-lysine. In this respect, they are closely related to corn, soy, and 2 blue-green algae. They differ from one of the known N-lysine proteins, lysozyme, in lacking valine in the N-penultimate position (5). They now appear to differ further from lysozyme in lacking C-leucine(9, Table I). The value of a quantitative subtractive N-terminal method for characterization of unfractionated proteins is demonstrated by these results. Conventional tagging procedures following fragmentation of unfractionated proteins would not permit conclusions on the sequential location of each type of amino acid.

The results with leucine indicate that the content of this amino acid in the hybrid is not intermediate between those of the parents. This relationship applies for the total content or that in the N-terminal position or in the N-penultimate position. The total lysine contents and the terminal lysine contents are, however, intermediate for the hybrid.‡ This result suggests that some amino acids, and/or some amino acids in particular positions in the protein portions of the organism, are more intimately involved with biological specificity than others§. Whether or not these

‡ The total N value is also intermediate.

§ This finding supports a similar earlier interpretation of the importance of basic amino acids, *cf.* Block, R. J., *Yale J. Biol. Med.*, 1935, v7, 235.

contents are fundamentally part of the genotype or part of the phenotype is of interest.

The answer to this question also bears on whether protein or nucleic acid is the heart of the genetic apparatus. Evidence can be cited for the importance of either type of structure (16,17). It is, therefore, possible that either protein or nucleic acid or both may function as ultimate determinants of biological specificity, or both may be manifestations of a more fundamental structure, or perhaps, interactions of structures. The present results, like those of Knight's study on tobacco mosaic virus proteins(16), indicate that amino acid-containing structures play a central role in some of such determinations. On the other hand, the limited diversity of types found in cereal seeds(7) by these criteria, as well as statistical analyses of amino acid composition(18), suggest that no very elaborate structural code would be required for the nucleic acids to determine types of protein.

Summary. Wheat, rye, and a wheat-rye hybrid have been analyzed for leucine, lysine, methionine, and phenylalanine. The assays have included total, N-terminal, N-penultimate, and C-terminal proportions of these amino acids. In all cases, only lysine is N-terminal. C-Terminal assays permit the conclusion that this lysine is all terminal, and not free. The proportions of total lysine and N-lysine in the hybrid are intermediate between the values in the parent strains. The comparable result for leucine is not intermediate.

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Latent Viral Infection of Cells in Tissue Culture. III. Role of Certain Amino Acids.* (22526)

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Previous investigations have demonstrated that tissue cultures of minced chick embryos, maintained for 11 to 14 days in a simple medium of inorganic salts and glucose, can be infected readily with psittacosis virus but do not support the multiplication of this agent (1,2). Following inoculation, such a latent infection can be maintained successfully for periods up to 15 days, the duration of cell survival under such conditions(2). Moreover, it has been established that viral multiplication can be induced at any time during this period by the addition of embryo extracts or

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