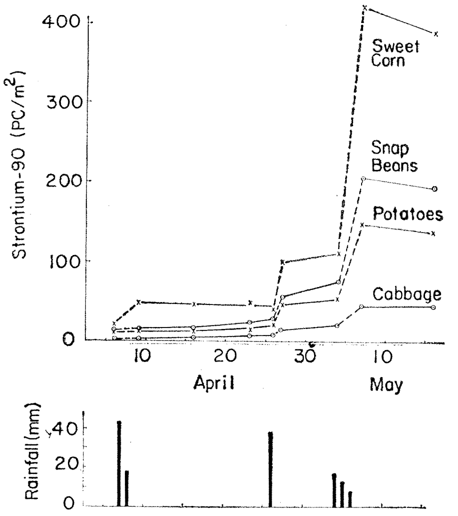

Fig. 1. Strontium-90 content in four crops grown at Belle Glade, Florida, in relation to date and amount of rainfall.

refined growth curve. We estimate that the average errors associated with the values in Fig. 1 are ± 12 percent for cabbage and sweet corn, and ± 15 percent for snap beans and potatoes. Strontium 90 content increased greatly during each interval with rainfall except when the crops were very small. There was comparatively little accumulation during intervals without rain. The changes in strontium-90 content between 7 and 16 May were smaller than the average errors associated with these contents.

The strontium-90 accumulation during rainy intervals may have resulted largely from interception of rain by the foliage and subsequent evaporation. Interception by a fully grown crop of corn may amount to 5 mm, increasing with increasing duration and decreasing intensity of rain (*2*). This amount would have been an appreciable fraction of the rains which fell separately on the afternoons of 4, 5, and 6 May, depositing a total of 410 pc of strontium per square meter. Less interception would have been expected in smaller crops, in agreement with the observations for other crops on the same date and for all crops on earlier dates. The earlier rains deposited a total of 310 pc of strontium-90 per square meter on 7 and 8 April, and 280 pc on 26 April.

Strontium-90 in rain which penetrated the crop cover would have been expected to enter the crop through its root system slowly over extended periods of time. The strontium-90 would have been absorbed on the soil within a few centimeters of the surface, and might have been taken up through shallow roots. Had this occurred with the Belle Glade crops, it should have continued as long as the surface soil moisture remained above the wilting point and should not have been confined to the interval during which rainfall occurred. We believe that penetration of strontium-90 through the crop cover, with subsequent uptake from the soil, was not an important pathway of contamination with these crops.

Any accumulation of strontium-90 in the crops between rains could have resulted either from uptake from the soil or from deposition on foliage. At Belle Glade, an accurate estimate of this accumulation was impossible. However, at Brawley all of the accumulation occurred without rainfall. The yield of dry matter and strontium-90 content increased quite uniformly throughout the sampling period. Although growth differed considerably at the two locations, the observed accumulation of about 25 pc of strontium-90 per square meter in the crops grown at Brawley was consistent with the possible accumulation between rains at Belle Glade.

Since the uptake of exchangeable calcium and strontium from soils are roughly proportional (*3*), the uptake of strontium-90 could be estimated from knowledge of the rooting habits and calcium contents of the crops. Each crop grown at Brawley took up about 0.2 percent of the exchangeable calcium in the top 35 cm of soil. The exchangeable strontium-90 content in the same layer of soil was 7000 pc/m^2 so that uptake of about 14 pc/m^2 would have been expected. Therefore, a substantial fraction of the observed strontium-90 content in the Brawley crops was derived from soil uptake. Similarly, the uptake of strontium-90 from the soil was about 37 pc/m^2 in the crops grown at Belle Glade.

It was possible that accumulation between rains arose from deposition of dust on foliage. Total deposition of strontium-90 in the rainfall collection pans at Brawley was 35 pc/m^2 and that between rains at Belle Glade was 84 pc/m^2. However, during dry periods there was no apparent correlation between increments of strontium-90 content in the crops and concurrent concentrations of strontium-90 in the air (from 0.044 to 0.095 pc/m^3 at Brawley and from 0.045 to 0.105 pc/m^3 at Belle Glade).

Thus it appears that the occurrence of rainfall was very important in the contamination of these crops by fallout. While there may have been some contamination from the atmosphere between rains, we could not determine the quantity in this experiment (*4*).

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26 August 1963

Steric Factors in the Chemistry of Polypeptides, Poly-α-Amino Acids and Proteins

Abstract. *An application of the rule of six for the estimation of steric hindrance in reactions of dipeptides and polypeptides, and conformational preferences of polypeptides and proteins, is described.*

Steric factors have a pronounced influence on the rates of hydrolysis of dipeptides and proteins (*1–3*), on the dependence of main chain conformation of proteins and polypeptides on amino acid sequence and composition (*4*), and on many other aspects of protein chemistry. The recognition of the importance of steric factors in organic chemistry has increased significantly in the past two decades (*5*).

The "Rule of Six" states: "In reactions involving addition to an unsaturated function containing a double bond, the greater the number of atoms in the six position the greater will be the steric effect." Numerous examples demonstrating the validity of this rule have been reported (*5*, chap. 4). Correlations also show that a given number of atoms in position 6 confer much greater steric hindrance (that is, they have a greater effect on rate constants) than in either position 5 or 7. Although the rule of six is fairly well known and widely used by organic chemists, it does not appear to have caught the attention of protein chemists and biochemists, at least in its broad aspects. The present report will point out the qualitative utility of the rule in the assessment of steric effects in the chemistry of polypeptides and proteins.

Table 1. Correlation of the rule of six with rates of hydrolysis of dipeptides (*1*) and polypeptides (*1*, chap. 4).

Peptide	Six number*	Relative rates of acid hydrolysis†
	Dipeptide	
Gly gly	0	1
Ala gly	0	0.62
Gly ala	3	.62
Gly leu	3	.40
Gly try	3	.35
Gly val	3	.31
Leu gly	3	.23
Leu leu	6	.048
Leu try	6	.041
Val gly	6	.015
	Polypeptide‡	
Polyglycine	0	500
Poly-DL-alanine	3	100
Poly-DL-α-amino butyric acid	6	22
Poly-DL-phenylalanine	5	13
Poly-DL-norleucine	6	20
Poly-DL-leucine	6	17
Poly-DL-isoleucine	9	5

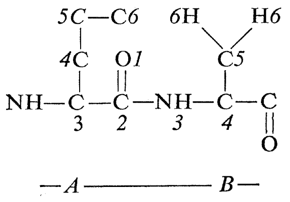
* Excluding O of C = O and OH in the carboxyl group of main chain, common to all the compounds. This is true for all six-number values discussed herein. † Gly. Gly = 1. Relative rates of hydrolysis in mixture of equal parts by volume of 10*N* HCl and glacial acetic acid. ‡ Polly-DL-alanine = 100. Relative rates of hydrolysis in 4*M* dichloroacetic acid.

Table 2. Contribution to peptide six numbers by various amino acids when located in either the *A* or *B* position of an -*A*-*B*-peptide sequence.*

Amino Acid	Number of atoms in position six		Number of heteroatoms in position six	
	A	B	A	B
Alanine	0	3	0	0
Arginine	3	3	0	0
Aspartic Acid	2	3	2	0
Glutamic Acid	3	3	0	0
Glycine	0	0	0	0
Histidine	2	3	1	0
Isoleucine	6	3	0	0
Leucine	3	3	0	0
Lysine	3	3	0	0
Methionine	3	3	1	0
Phenylalanine	2	3	0	0
Proline	6	6	0	0
Serine	1	3	0	1
Threonine	4	3	0	1
Tryptophan	2	3	1	0
Tyrosine	2	3	0	0
Valine	6	3	0	0
Cysteine	1	3	0	1
Cystine	1	3	1	1
Cysteic acid	3	3	3	1

* The six number for the peptide bond between any two amino acids is obtained by adding the appropriate numbers in columns A and B. For example, the six sn for Val Ala would be 9 (6 plus 3).

The number system used for a peptide bond is illustrated as follows:

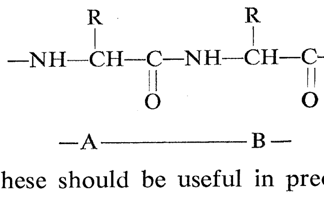


I

Correlation of the rule of six with rate data, determined by Synge (*1*), for the acid hydrolysis of a series of dipeptides and for acid hydrolysis of polypeptides (*2*) is shown in Table 1. The number of atoms in position six is called the "six number." Those dipeptides with a six number of 0 hydrolyze fastest; those with a six number of 3, at intermediate rates; and those with a six number of 6, slowest. The spread in rates covers a factor of about 60. The same correlation of rates with six numbers is found in hydrolysis of the polypeptides.

That valyl peptides resist hydrolysis has been known for a long time (*3*, *6*). The valyl group exerts a stronger influence when attached to the carbonyl side of a peptide bond than when bonded to the NH; attached to the carbonyl side it has a six number of 6 compared with a six number of 3 in the latter position. Valylvaline with a six number of 9 is exceptionally stable, being isolated in 5 to 6 percent yield from gramicidin after 6 hours in boiling 16 percent HCl, and in 1.5 percent yield after 24 hours under the same conditions (*6*).

The six numbers have been calculated (Table 2) for the peptide bonds between *A* and *B* of all possible -*A*-*B*-peptide sequences involving the more commonly occurring α-amino acids.



$$-\mathrm{NH}-\overset{\mathrm{R}}{\overset{|}{\mathrm{CH}}}-\underset{\mathrm{O}}{\underset{\|}{\mathrm{C}}}-\mathrm{NH}-\overset{\mathrm{R}}{\overset{|}{\mathrm{CH}}}-\underset{\mathrm{O}}{\underset{\|}{\mathrm{C}}}-$$

II

These should be useful in predicting the relative stabilities of peptide bonds in proteins and related substances to hydrolysis, hydrazinolysis, alcoholysis, and related reactions centered at the peptide bond; particularly so for peptides in which *A* and *B* are derived from alanine, glycine, isoleucine, leucine, phenylalanine, proline, or valine, since for this group of amino acids, steric factors are probably more important than other factors, there being no heteroatoms in the side chain to provide neighboring group participation in main chain cleavages.

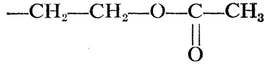
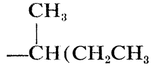
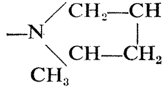
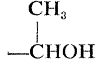
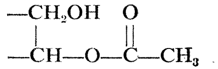
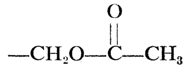
Many peptide units contain heteroatoms in the side chain. The presence of -OH, -COOH, -SH, and -SO$_3$H, in the side chain of a peptide unit is known to accelerate the rate of cleavage of peptide bonds flanked by these groups (*2*, *7*). The extent of the neighboring group participation may be greatly modified by steric factors, being less for peptide bonds with higher six numbers. Not all serine and threonine peptide bonds are equally reactive in hydrolysis of various proteins (*8*).

Six numbers are also useful when considering steric factors about peptide bonds in modified proteins, derivatives, and related compounds. Although one can always calculate the six number for a given peptide, lists such as Table 2 are convenient and facilitate comparisons. For proteins of known amino acid sequence, a listing of the six number for each peptide bond along the sequence gives a steric topography for the molecule.

The six numbers are intended to give only a qualitative estimate of the relative importance of steric factors; they are particularly useful for those reactions in which the intermediate or activated complex has a tetrahedral configuration. Molecular models will give a more lucid picture of the steric condition about a specified peptide bond and should be used, for confirmatory purposes. Examination of molecular models will make quite clear the reason why larger six numbers tend to provide greater steric hindrance. The greater the number of atoms in position six, the more difficult it becomes to remove a blocking group from the reaction path, since rotation of a given group out of the path merely results in rotation of a new blocking group into the path. With six numbers of 9, 12, and more it becomes difficult, if not impossible, to obtain a molecular conformation with no group blocking the reaction site.

Six numbers of peptides may also be useful in other stereochemical problems, including qualitative estimation

Table 3. Conformational classification of α-amino acids and six numbers of peptide units containing these (*4*).

α-Helix-forming amino acids	R	*Six numbers* of peptide bond (See II)	
		Total	Heteroatoms in position six
Alanine	$-CH_3$	3	0
Aspartic Acid Esters	$-CH_2COO$ Alkyl	6	2
Glutamic Acid	$-CH_2CH_2COOH$	6	0
Glutamic Acid Esters	$-CH_2CH_2COO$ Alkyl	6	0
Leucine	$-CH_2CH-(CH_3)_2$	6	0
Lysine	$-(CH_2)_4-NH_2$	6	0
Methionine	$-CH_2CH_2SCH_3$	6	1
Phenylalanine	$-CH_2-$	5	0
Tyrosine	$-CH_2-$ $-OH$	5	0
Norleucine	$-CH_2-CH_2-CH_2CH_3$	6	0
Norvaline	$-CH_2-CH_2CH_3$	6	0
o-acetylhomoserine	 $-CH_2-CH_2-O-\underset{\underset{O}{\Vert}}{C}-CH_3$	6	0
Valine	$-CH(CH_3)_2$	9	0
Isoleucine	 $-\overset{\overset{CH_3}{\vert}}{C}H(CH_2CH_3)$	9	0
Proline		12	0
Threonine	 $-\overset{\overset{CH_3}{\vert}}{C}HOH$	7	1
o-acetyl threonine	 $-\overset{\overset{-CH_2OH}{\vert}}{C}H-O-\overset{\overset{O}{\Vert}}{C}-CH_3$	7	1
Serine	$-CH_2OH$	4	1
o-acetyl serine	 $-CH_2O-\overset{\overset{O}{\Vert}}{C}-CH_3$	4	1
s-methyl cysteine	$-CH_2-S-CH_3$	4	1

of the effect of steric factors on the main chain conformations of polypeptides and proteins, the tendency of these macromolecules to crystallize, and the tendency for hydrophobic bonds to form. Six numbers have not been suggested previously, however, for use in such problems, but they have possible applications.

For example, it has been proposed that the amino acid sequence of polypeptides and proteins has an influence on main-chain conformations and hence, at least indirectly, exerts some influence on secondary and tertiary structure of proteins. Blout (*4*) has reviewed the status of work of this type. The α-amino acids are divided into two groups: those that tend to favor and permit α-helix formation and those that favor the β- and random coil conformations and appear not to form an α-helix. This classification is based on results obtained with synthetic polypeptides. Whether it will also apply to proteins remains to be shown. Amino acids are listed in Table 3, along with the six number for peptide bonds in homopoly-α-amino acids of each. It will be noted that for α-amino acids with no heteroatoms in position 6 of the side chain (with the exception of methionine and aspartic esters), those with a six number greater than 6 do not favor α-helix formation. Also, the presence of a heteroatom in position six works against α-helix formation. Blout has made the generalization that amino acids not forming the α-helix include those with either a heteroatom or with disubstitution on the β-carbon atom. As will be noted from the previous discussion, disubstitution on the β-carbon atom results in high six numbers; a heteroatom on the β-carbon atom will be in position 6 in the *B* location of an *-A-B-* peptide, in the 5 position in the *A* location. Six numbers should prove particularly useful in predicting the preferred conformations of various copolypeptides and of proteins of known sequence. For example, an alternating copolymer of valine and glycine would have peptide bonds with six numbers of 6 and 3 and would exist in the α-helix conformation, whereas an alternating copolymer of valine and alanine would have six numbers of 9 and 3 and would not prefer the α-helix conformation (there is a large steric hindrance to intramolecular H-bond formation in such cases). By assigning each amino acid in a copolypeptide a value proportionate to its molar ratio in the molecule and its own six number, the average six number for a peptide bond of the copolymer can be estimated, and the probable chain conformation "guessed at." The utility of six numbers for this purpose remains to be demonstrated.

Likewise, six numbers should prove useful in making a qualitative estimate of the tendency towards hydrophobic bond formation at specific locations along a polypeptide chain. This tendency should be greater at positions with higher six numbers, since the higher the six number the greater the local intramolecular concentration of hydrophobic groups.

In applying six numbers to polypeptide and protein chemistry, other factors will contribute simultaneously—electrostatic and dispersion forces, H-bonding, *cis-trans* isomerism about peptide bonds, and absolute configuration of the asymmetric carbon. This qualitative method is not a substitute for the more difficult and more quantitative treatments in which the actual magnitude of steric energies is estimated to the best approximation.

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3 June 1963

Suspended Clay in a Water Sample from the Deep Ocean

During a recent cruise of the research vessel *Conrad* from New York to Martinique an attempt was made to recover particulate matter in suspension in ocean water. Fifteen samples of 3000 liters each from a depth of 3 meters were processed by pumping water aboard and separating suspended