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Dialysis Studies. VII. The Behavior of Angiotensin, Oxytocin, Vasopressin, and Some of Their Analogs*

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The technique of thin-film dialysis has been used to study the conformations in aqueous solution of angiotensin II, oxytocin, vasopressin, and some of their analogs. By using membranes of high selectivity it was found that angiotensin II exists in a coiled form of low axial ratio. Oxytocin has a compact conformation with the tripeptide tail held close to the ring, whereas vasopressin has a more extended conformation because of the repulsion between the basic groups in the ring and the tail. Small differences in diffusion rate were detected among some of the analogs studied. The effect of ammonium acetate on some of the peptides was determined.

Now that many of the difficulties encountered in the isolation and precise characterization of a number of the smaller polypeptide hormones have been successfully overcome it seems of some interest to reconsider the particular properties that have contributed to these difficulties and also other properties of primary interest to the separation problem. These include properties which determine stability, adsorbability, diffusibility, partition behavior, solubility, association behavior, and detectability by quantitative biological assay. While much has been learned in the course of the isolation studies by trial and error it is immediately evident that much further information is needed before a precise correlation of properties and structure can be made. The properties of interest mentioned above are probably the same as those which will ultimately prove to be of the greatest interest in understanding the physiological role of the substances.

Thin-film dialysis (Craig and King, 1962) is a technique which can well give information about many of the properties in question. It offers direct information particularly about diffusibility in different solvent environments and at more than one temperature. This information can be interpreted in terms of the association of an individual molecule with others of its own species or association with the solvent molecules. When the chain length of polypeptides is sufficiently great intrachain association or interaction can also take place and a balance of interactions is involved. A study of diffusibility under different conditions can give information (Craig, 1962) about overall changes which take place in the conformation.

The structural formulas for angiotensin (Skeggs *et al.*, 1956; Elliot and Peart, 1956), oxytocin (Du Vigneaud *et al.*, 1953b, 1954), and 8-lysine vasopressin (Du Vigneaud *et al.*, 1953a, 1957) have been determined by degradation and synthesis (Fig. 1). In addition many analogs of these substances are now being synthesized and their physiological actions are being studied in an attempt to determine the features of the structure which are required for the physiological action. In view of these developments, it has appeared a particularly appropriate time to study a series of these peptides by the dialysis technique. Some of our experience gained thus far is reported in the present paper.

Experimental

The type of diffusion cell employed in this study was the type previously reported (Craig and Konigsberg, 1961) with the removable center tube. The cellophane membranes were all prepared from Visking dialysis casing.

The tetrapeptide, the hexapeptide, angiotensin II, angiotensinamide[1] and angiotensin I all were synthetic peptides (Schwyzer, 1958) obtained from Dr. R. Schwyzer of the Ciba Company. The heptapeptide was a synthetic preparation (Shields and Carpenter, 1961) obtained from Dr. F. H. Carpenter of the University of California. It is the peptide which includes residues 23–29 of the B chain in insulin. The bradykinin was a synthetic peptide obtained from Dr. M. Bodanszky of the Squibb Research Institute. Oxytocin, vasopressin and their analogs were all obtained

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[1] Abbreviations used in this work: angiotensinamide, β-aspartylamide of angiotensin II; ACTH, adrenocorticotropic hormone.

from Dr. V. du Vigneaud of Cornell University Medical College except *N*-carbamyl oxytocin which was obtained from Dr. D. Smyth of the National Institute for Medical Research, Mill Hill. The preparations listed above were gifts from the various individuals mentioned. We wish to thank them for their interest and generosity. All the substances studied gave essentially a linear escape plot indicating a high degree of purity.

The rotatory dispersion measurements were made in the Rudolph spectropolarimeter Model 220.

Results and Discussion

Before discussing the experimental results presented in this paper a brief discussion of the limitations and possibilities of thin-film dialysis may be in order. Obviously the rate of diffusion of a solute through a membrane is a function of its size or perhaps more correctly of the volume the molecule occupies in solution. Judging from free diffusion studies diffusion through a membrane would also be a function of the molecular shape since a sphere would be expected to diffuse more rapidly than a rigid rod in which random rotational motion would make the effective diffusional volume considerably larger. At present, however, good data supporting speculations on the effect of shape, though available for free diffusion, are not available for the type of restricted diffusion taking place through membranes where the pores are only slightly larger than the diffusing solute. It is hoped that the type of study reported here will eventually throw light on this subject.

In a parallel study soon to be reported (L. C. Craig and E. J. Harfenist, paper in preparation) on much longer peptides with no covalent cross-links it has been found that their rate of diffusion through a calibrated membrane is much more rapid in 0.01 N acetic acid than in ammonium acetate. For instance, pork ACTH dialyzes at a relatively slow rate when the solvent is an ammonium acetate buffer in which the diffusion coefficient as determined in the ultracentrifuge is found to be low. Since in this buffer data from the ultracentrifuge obtained by the equilibrium method have shown the state of the molecule to be essentially monomeric, it follows that the low diffusion coefficient must be due to a high axial ratio of the order of 7:1 as postulated by Squire and Li (1961) in their interesting study of the association of ACTH. However, by use of 0.01 N acetic acid in place of the buffer the dialysis rate can be increased by a factor of 13. The shape of the molecule must therefore be much less extended at low ionic strength in 0.01 N acetic acid.

In spite of the need for more data in regard to the shape factor, the data thus far at hand (Craig and King, 1962; Craig, 1962; Craig and Pulley, 1962) are overwhelmingly in favor of an overall size factor combining volume and shape as the predominant molecular characteristic controlling the rate of dialysis through a given cellophane membrane. Misgivings concerning the role of fixed charges on the membrane have been effectively dispelled by potentiometric data indicating a very low level of fixed charge on the membrane (Craig and Ansevin, 1963) and by the fact that in a series of proteins the rate of escape has been clearly a function of size (Craig *et al.*, 1957) irrespective of the isoelectric point of the protein. This has been shown to be so even with the amino acids (Craig and Ansevin, 1963), where the charge on the solute would have the maximum effect in relation to size differences.

The evidence in favor of the size factor was greatly strengthened by the correlation of relative diffusion coefficients and relative escape rates in a series of sugars (Craig and Pulley, 1962). In studies using the Schardinger dextrins as models it could be deduced that an increase of a few per cent in the effective diameter of a sugar would be recognizable by means of the dialysis technique if a membrane of high selectivity should be employed.

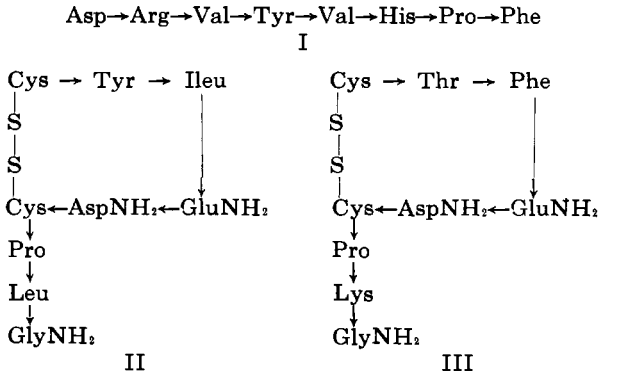

FIG. 1.—Structures of angiotensin II (I), oxytocin (II), and 8-lysine-vasopressin (III).

However, one disadvantage of the technique is that high selectivity is achieved only when the porosity of the membrane is adjusted so that it will barely permit the solute of interest to pass (Craig and Konigsberg, 1961). Thus a membrane is selective only for a rather narrow range of sizes, a range of the order of a factor of two or even less. We have found it convenient to base our conclusions on a 50% escape rate of several hours but not longer than 24 hours. Thus many different calibrated membranes are required for studying a wide spectrum of molecular sizes.

Unfortunately the acetylated membranes of low porosity have not proved as stable as desired for prolonged use. One may develop a pinhole without warning. Thus at the end of a few runs the membrane must be recalibrated with the same solute originally used for calibration in order to demonstrate that a change has not taken place. Exact matching of porosities with the membranes of low porosity and highest selectivity has proved to be a difficulty. It therefore seems best to draw conclusions on the basis of a series of comparative runs as closely controlled as possible but with the understanding that a certain variation will be encountered.

The data in Table I were all obtained with a single membrane calibrated afterward to demonstrate that its porosity had not changed. The order of the escape rates in this series was found to be that expected on the basis of molecular weight except for peptide 3, a peptide derived from the tryptic digestion of hemoglobin. The value for the hexapeptide does not agree with studies (Craig, 1962) published earlier. The reason for this is not now apparent since the earlier work involved several solvent systems. It may have been due to changed membrane porosity or to bacterial contamination of the membrane. Peptides of this type are very susceptible to the action of hydrolytic enzymes. The possibility of bacterial contamination, however, is reduced by the use of 0.01 N acetic acid as the solvent.

A small difference was noted between angiotensinamide and angiotensin II itself. This could indicate that the conformation of the amide is a little more extended. A similar difference was noted in careful experiments (Craig and Ansevin, 1963) with free aspartic and glutamic acids and their amides. Moreover, angiotensin II, but not the amide, shows a small shift

TABLE I
COMPARATIVE ESCAPE RATES OF ANGIOTENSIN ANALOGS

No.	Compound	Mol Wt	$T/2$ at 40° (hr) 0.01 N HAc	$T/2$ at 40° (hr) 0.01 N HAc, 0.15 M NH_4Ac pH 5.58
1	Tetrapeptide Val-Tyr-Val-His	517	1.5	
2	Hexapeptide Val-Phe-Val-His-Pro-Phe	745	3.7	
3	Val-Asp-Pro-Val-AspNH_2-Phe-Lys	818	6.8	
4	Heptapeptide Gly-Phe-Phe-Tyr-Thr-Pro-Lys	859	4.2	5.9
5	Angiotensin II[a] Asp-Arg-Val-Tyr-Val-His-Pro-Phe	1032	6.0	7.7
6	Angiotensinamide AspNH_2-Arg-Val-Tyr-Val-His-Pro-Phe	1031	8.0	8.0
7	Bradykinin Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1060	7.7	8.1
8	Angiotensin I Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu	1282	9.3	

[a] The terminal aspartic is β-linked.

on adding ammonium acetate. After these measurements were made it was learned (Riniker *et al.*, 1962) that this preparation of angiotensin II is different from the naturally occurring one. The alpha-peptide linkage of the amino-terminal aspartic acid has isomerized to the beta linkage. It is interesting that bradykinin with three proline residues is not out of line in this series if the peptides are listed in the order of increasing molecular weights. An analog of angiotensinamide in which the tyrosine residue was replaced by —NHCH-($CH_2C_6H_4OH$)—NHCO— was compared with the angiotensinamide. The 50% escape times were 14 and 10.6 hours, respectively. We wish to thank Dr. B. Riniker of the Ciba Company for this analog.

In a concomitant study with peptide fragments isolated after trypsin digestion of the separated chains from hemoglobin, it has been found that with a single exception thus far attributable to a particular distribution of positive and negative charges along the chain, a similar correlation of molecular weight with the rate of diffusion through the membrane can be established when 0.01 N acetic acid is used as the solvent (Craig and Harfenist, 1964). The peptides in Table I, except possibly no. 3, apparently assume a shape as nearly spherical as possible in this solvent but most of them show a somewhat reduced rate of diffusion when the ionic strength of the solvent is increased. The fact that some peptides show a large shift while others seem to be unaffected by the change in ionic strength (L. C. Craig and E. J. Harfenist, paper in preparation) indicates that the major effect stems from a change in the shape or compactness of the peptide rather than a change in the membrane. Studies with proteins (Craig, 1962; Craig *et al.*, 1958) are also in line with this viewpoint. An increase in the dielectric constant of the solvent should reduce the attraction between acid and basic groups along the chains of a peptide or protein and thus promote expansion. Peptides 6 and 7 showed little or no shift with ammonium acetate. Peptide 3 showed a shift in experiments with another membrane. The moderate shift noted with angiotensin II was much more pronounced at 25°. The shift seems to be caused by a change in electrostatic interaction of positive and negative charges removed from each other by several amino acid residues.

The data in Table II were obtained at a different time and with a different membrane from those in Table I. This membrane was a little less porous as shown by the half-escape time of 7 hours for the heptapeptide whereas in Table I the half-time was 4.2 hours for this peptide. Thus it would seem that oxytocin in 0.01 N acetic acid is a slightly smaller or more spherical molecule than angiotensin II even though they have the similar molecular weights, 1007 and 1032, respectively. The difference, substantiated in Table III, could result from a difference in conformation or from the fact that oxytocin has only one basic group at this pH whereas angiotensin has three and therefore could be more hydrated. From the membrane diffusion results with amino acids (Craig and Ansevin, 1963) the basic amino acids seem to be larger probably in part because of the anion and the water of hydration of the basic ion. As discussed later the peptide side chain of oxytocin is not extended. When this is taken into account the diffusional behavior of angiotensin in neutral or acid solution is consistent only with a compact molecule of near minimal axial ratio. This is in agreement with the spiral structure proposed by Smeby *et al.* (1962) for isoleucyl angiotensin II on the basis of the groups required for biological action, interpretation of rotatory dispersion data, and other considerations.

TABLE II
COMPARATIVE ESCAPE RATES OF OXYTOCIN ANALOGS

Compound	Mol Wt	Solvent	$T/2$ at 40° (hr)
Oxytocin	1007	HAc	9.0[a]
Oxytocin	1007	NH_4Ac	12.0
Deamino-oxytocin	992	HAc	9.6
9-Sarcosine-oxytocin	1021	HAc	14.0
1-Hemi-D-cystine-oxytocin	1007	HAc	15.3
1-(Hemi-homocystine)-oxytocin	1021	HAc	14.5
Oxytocin dimer	2014	HAc	33.0
Heptapeptide[b]	859	HAc	7.0
Bacitracin	1422	HAc	24.0

[a] This value is a comparative one derived from several runs with other membranes. [b] This is the same peptide as no. 4 in Table I.

The flexibility of the angiotensinamide molecule is indicated by its behavior in 0.1 N ammonium hydroxide. Here the escape rate was retarded by a factor of 4. Studies in the ultracentrifuge carried out by Dr. David Yphantis have indicated that angiotensinamide is not appreciably associated throughout

TABLE III
COMPARATIVE ESCAPE RATES OF OXYTOCIN AND VASOPRESSIN ANALOGS

Peptide	$T/2$ (hr)	No. of Separate Runs
Angiotensinamide	3.6	3
Oxytocin	3.2	5
8-Lysine-vasopressin	5.3	3
8-Histidine-vasopressin	4.5	1
8-Lysine-vasotocin	5.2	1
1-Deamino-8-lysine-vasopressin	3.1	3
1-Deamino-oxytocin	3.5	2
1-Acetyl-8-lysine-vasopressin	4.1	2
1-*N*-Methyl-oxytocin	4.1	1
4-Decarboxamido-oxytocin	3.8	1
D-Leucine-oxytocin	3.7	2
4-Deamido-oxytocin	2.6	2
Oxytocin dimer	9.8	1

the alkaline *p*H range. Accordingly it is probable that the molecule becomes considerably more extended in alkaline solution. Even 0.001 M ammonium carbonate was found to reduce greatly the rate of diffusion.

It seems, therefore, of considerable interest that the hormone has been found to be more active (Paladini *et al.*, 1963) when injected into rats at an alkaline *p*H and that a similar potentiating effect of the alkaline *p*H has been demonstrated on the stimulating action of angiotensin on smooth muscle (Huidobro and Paladini, 1963). This raises the question as to whether or not the extended conformational form favored by ammonium hydroxide could have a degree of stability. In order to test this possibility the peptide dissolved in 0.1 N ammonium hydroxide was placed inside the membrane. Dialysis against distilled water now did not give an escape rate more rapid than when the peptide was dialyzed against 0.1 N ammonium hydroxide. After dialysis for some hours the *p*H inside the membrane remained on the alkaline side indicating that ammonia remained bound. This behavior and the increased activity bring to mind the behavior of ACTH (Li, 1958) which becomes nondialyzable and shows an increased activity after treatment with dilute ammonium hydroxide. In the latter case, however, association to a higher molecular weight seems to be involved.

The effect of urea was also investigated. With angiotensinamide a moderate retardation in escape rate was noted at 25° when the solvent, 0.01 N acetic acid, was made 6 M in urea. This shift is interesting in the light of the experience of Bumpus *et al.* (1961) who found that urea and arginine inactivated angiotensin II whereas these solutes had no effect on oxytocin. They ascribed this difference to the fact that angiotensin II is not rigidly held in a particular conformation but can assume an inactive form whereas oxytocin is restricted because of the ring structure. When angiotensinamide was first dissolved in 6 M urea and then dialyzed against 0.01 N acetic acid the escape rate was the same as if no urea had been used. Apparently the shift is rapidly reversible.

Smeby *et al.* (1962) pursued the idea of a conformational shift further by studying the change in rotatory dispersion caused by urea. It was not large. We also have measured the rotatory dispersion shift caused in angiotensinamide. In our study a simple type of rotatory dispersion fitting quite well the one-term Drude equation was found. The constant, λ_c, was found to be 202 mμ in water, 202 mμ in 6 M urea, 187 mμ in 0.01 N acetic acid, 220 mμ in 0.1 N ammonium hydroxide, and 207 mμ in 0.01 N acetic acid–0.15 M sodium chloride. Paiva *et al.* (1963) have recently studied the rotatory dispersion and other properties of angiotensinamide. They concluded that it should have a random conformation rather than that of a fixed helix.

The changes noted in the membrane diffusion study are apparently more striking than those noted by rotatory dispersion and probably can be related to overall shape more reliably. The diffusion data are not against the helical conformation proposed by Smeby *et al.* (1962). To the contrary, they could be interpreted as indicating a rather spherical conformation of near-minimal size. Many peptides studied by the thin-film dialysis technique have shown a much larger shift when salt is added. This lack of effect when the dielectric constant is altered could indicate only a weak electrostatic interaction between the basic amino terminus and the carboxyl terminus. The latter could be partially satisfied at the lower *p*H by the proximity of the imidazole of the histidine.

On the other hand, 0.1 N ammonium hydroxide could expand the molecule by discharging both the terminal-amino charge and that of the imidazole and disruption of the possible interaction between the aromatic rings of the phenylalanine and tyrosine by ionization of the phenolic group. The apparent binding of the ammonium ion noted above could indicate a degree of stability for another conformation and thus explain the enhanced biological activity in rats. The expanded conformation produced when angiotensin is treated with urea either with or without concomitant binding of urea could explain the mysterious inactivation noted by Bumpus *et al.* (1961).

Recently definite evidence has been presented (Craig *et al.*, 1963) showing that more than one conformational form of ribonuclease will result depending on the conditions of isolation. In the proper environment, the forms are slowly interconvertible. The present study contributes to the possibility that similar subtle effects may be found even with relatively short polypeptides.

A number of the differences in Table II are rather striking. However, before placing too exact an interpretation on the data it might be well to state that further checking of these particular results would be desirable. They represent single experiments with very costly preparations and were made with the minimal amount of material consistent with a significant result.

The appreciable change in the dialysis rate of oxytocin on addition of ammonium acetate could indicate an expansion due to the change in dielectric strength of the solution. It is interesting that replacement of the amino group with hydrogen (deamino-oxytocin, Du Vigneaud *et al.*, 1960) results in a molecule which diffuses at almost the same rate, but that addition of a methyl group to the amino group of the carboxy-terminal glycine residue (9-sarcosine-oxytocin, Cash *et al.*, 1962) definitely results in a more slowly diffusing compound. When the ring is enlarged by a single carbon atom (the hemi-homocystine derivative, Jarvis *et al.*, 1961), it might be expected that a measurably slower rate would result, as was observed; but the result obtained when only the configuration of a single carbon atom is changed (the 1-hemi-D-cystine derivative, Hope *et al.*, 1963) was not expected. However, in such a tightly packed ring structure the configuration of each residue may be very important.

The dimer of oxytocin (Ressler, 1958) diffused much more slowly than the other derivatives. It was also compared at 40° with bacitracin and gramicidin S (Craig *et al.*, 1950) in a more porous membrane in

which the dimer gave a half-escape time of 7.5 hours. The comparative rates were 7.5, 4.7, and 11 hours, respectively. The ring of the oxytocin dimer contains 12 amino acid residues where as that of gramicidin S contains 10. The faster dialysis rate of the dimer would seem to indicate that it has some degree of folding. The slow rate for the diffusion of gramicidin S indicates this cyclic peptide to have an open type of conformation probably of near maximum size. This is not in agreement with the speculations of Warner (1961).

From the rather limited data of Tables I and II it appears that small changes in structure have a greater influence on diffusional properties in the oxytocin series than in the angiotensin series. This might be expected because the ring in oxytocin severely limits the conformational adjustments which may well be possible in a linear peptide. In the latter case the actual shape of the molecule may be a statistical average of equilibrium forms to give an average overall axial ratio influenced by the solvent.

After the rather preliminary data in Table II had been obtained it seemed advisable to undertake a much more careful and extensive study of the valuable synthetic oxytocin and vasopressin analogs. In this second series comparisons were made by repeated runs so that the small differences would have more significance. In Table III the number of separate runs are given. These data were all obtained in 0.01 N acetic acid at 40° with two membranes which happened to have identical porosities, at least within the experimental error. In addition comparisons were made in several less porous membranes to substantiate further some of the smaller differences. These are not listed in the table but give the results strong further support.

The most interesting information to be derived from Table III is the slower rate of dialysis of 8-lysine-vasopressin as compared to oxytocin. In a recent paper (Craig and Harfenist, 1964) it has been shown that, where there is more than one charge spaced appropriately on a peptide chain, charge interaction will strongly affect the conformation. In the case of vasopressin the repulsion of the two positive groups would be expected to cause the peptide side chain to be extended. Since oxytocin was consistently found to dialyze more rapidly its peptide side chain must be folded closer to the ring and held there perhaps by hydrogen bonding or other forces. In support of this 8-histidine-vasopressin (Katsoyannis and Du Vigneaud, 1958) and 8-lysine-vasotocin (Kimbrough and Du Vigneaud, 1961) both dialyze at a rate similar to that of vasopressin. Since the ring in vasotocin is the same as that in oxytocin the slower rate cannot be ascribed to the phenylalanine in vasopressin. The difference must be in the side chain. Moreover, 1-deamino-8-lysine-vasopressin (Kimbrough *et al.*, 1963) dialyzes at nearly the same rate as oxytocin and 1-deamino-oxytocin. When the 1-amino group of vasopressin is acetylated (Cash and Smith, 1963) the rate of dialysis is increased, but not as much as by complete removal of the amino group. In both these derivatives removal of the basic group should favor a more compact molecule.

1-*N*-Carbamyl-oxytocin (Smyth, 1964) was found to dialyze only slightly more slowly than oxytocin ($T/2$ values of 8.2 and 7 hours). When the amino group of oxytocin is methylated (V. V. S. Murti, W. D. Cash, L. A. Branda, and V. Du Vigneaud, 1964, unpublished data) a slightly slower rate of dialysis results as is the case when the 4-carboxamide or 5-carboxamide group (compared in a less porous membrane) is removed (Du Vigneaud *et al.*, 1963). D-Leucineoxytocin (Schneider and Du Vigneaud, 1962) shows only a slightly slower rate of dialysis than does the L-diastereo isomer. It is interesting that these two isomers could be separated by countercurrent distribution. When the amide group of the glutamine residue in oxytocin is removed (4-deamido-oxytocin, Photaki and Du Vigneaud, 1964) the rate appears to be slightly accelerated as might be expected from the possibility of charge interaction of the positive and negative charges.

The dialysis rate of 8-lysine-vasopressin was found to be nearly the same in 0.01 N acetic acid or in 0.15 M ammonium acetate–0.01 N acetic acid buffer at *p*H 5.58. The rates of oxytocin, 1-deamino-8-lysine-vasopressin, and 1-acetyl-8-lysine-vasopressin were appreciably retarded in the buffer.

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Isolation of N^6-(Aminoacyl)adenosine from Yeast Ribonucleic Acid*

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A group of compounds with the general structure, N^6-(aminoacyl)adenosine, I, has been isolated from the soluble RNA fraction of yeast. The aminoacyladenine bond is stable to 0.5 N hydrochloric acid at 100° for 2 hours, to alkaline buffer (*p*H 10.5) at room temperature for 24 hours, and to 2.0 M hydroxylamine (*p*H 7.0) at room temperature for 3 hours. The following amino acids have been found in this group: alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, and valine, of which aspartic acid, glycine, threonine, and valine predominate. Although the majority of these compounds appear to consist of adenosine and a single amino acid, the possibility exists that some of these compounds consist of adenosine and a polypeptide chain.

Several reports have appeared in the literature which describe the isolation of polypeptides and amino acids linked covalently to nucleotides.[1] These compounds have been found in both the acid-soluble extracts and the RNA of a number of tissues and, according to the nature of the covalent bond between the amino acid and nucleotide, may be classified into three groups. These classifications are as follows:

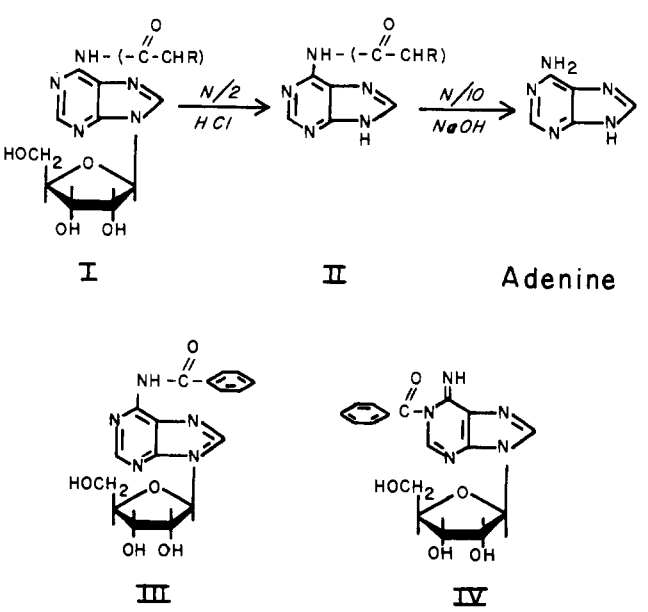
Phosphoanhydride Linkage.—Polypeptidyl-nucleotides, in which the carboxyl group of the terminal amino acid of the polypeptide chain is attached to a phosphate residue of the nucleotide in an anhydride bond, have been isolated from yeast (Harris and Wiseman, 1962; Schuurs and Koningsberger, 1960).

Ester Linkage.—Amino acids are attached to transfer RNA by esterification of the 2′- or 3′-hydroxyl of the terminal adenosine unit (Zachau *et al.*, 1958; Preiss *et al.*, 1959). Habermann (1959) and De Kleot *et al.* (1960) have shown that yeast and mammalian RNA contain firmly bound polypeptides. They suggest that as in the above case the carboxyl end of the polypeptide chain is linked to the 2′- or 3′-hydroxyl group of the nucleoside residue. Harris and Wiseman (1962) have isolated small oligonucleotides from yeast in which polypeptides are similarly bound.

Phosphoramide Linkage.—Potter and Dounce (1956) isolated nucleotide fractions from the RNA of yeast, calf pancreas, and rabbit liver which contained firmly bound amino acids and short polypeptides. On the basis of their evidence they suggested that these compounds are bonded between the phosphate residue of the nucleotide and an available amino group of the peptide chain. A more recent report (Bogdanov *et al.*, 1962) presents similar evidence for the existence of such nucleotide-peptide complexes. A considerable body of information has thus accumulated concerning the occurrence of amino acid- and peptide-nucleotides in nature. The purpose of the present paper is to report the isolation of a hitherto unrecorded type of aminoacyl-nucleoside which, on the basis of chemical evidence, has been assigned the structure, N^6-(aminoacyl)adenosine, I.

EXPERIMENTAL

Yeast s-RNA (22 g, 70% pure) was prepared for us by General Biochemicals according to the method



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[1] Many of the earlier references have been summarized in a paper by Wilken and Hansen (1961).