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## Polarographic Studies on Natural Peptides. I. Polarography of Oxytocin, Lysine, and Arginine-vasopressin in Cobalt(II) Ammonia–Ammonium Chloride Solutions[1a]

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The polarographic behavior of the natural peptides, oxytocin, lysine-vasopressin and arginine-vasopressin, in cobaltous ammonia–ammonium chloride solution has been studied. These were selected as model compounds to learn more concerning the nature and origin of the so-called "protein waves." Both bis-(acetyl)-lysine-vasopressin and S,S'-bis-(benzyl)-lysine-vasopressin were prepared and isolated by countercurrent distribution to prove the origin of certain polarographic waves for the original lysine-vasopressin. Parameters studied with respect to their effect on the polarographic pattern were peptide concentration, pressure on the dropping mercury electrode, variations in ammonia concentration and the effect of peptide concentration on the half wave potential of cobaltous ion.

In 1933 Brdicka[2] used serum in order to suppress the cobalt maximum wave in cobaltous ammonia–ammonium chloride solution. Although the maximum wave was suppressed, he discovered two new waves which followed the cobalt reduction step. A somewhat similar pattern was obtained in cobaltic solutions. In order to explain these unexpected phenomena, he investigated the proteins which produced the two new waves ("protein double wave"). Brdicka suggested that the protein double wave may depend on the different amino acids in the protein. Cysteine and cystine showed one maximum wave (referred to as a "cystine wave") at a potential similar to the second maximum wave of the protein double wave. The cystine wave also differed in that it was obtained only in cobaltous solutions. Other amino acids did not show such a wave.[3] In fact, proteins containing no cystine or cysteine did not show the protein double wave. Therefore, it was concluded that protein–SH or –S–S– produced the protein double wave. However other compounds containing –SH, –S–, or –S–S–, such as thioglycolic acid, mercaptides, di-(2-chloroethyl)-sulfide, thiamine, etc., also show a similar polarographic catalytic wave in cobalt ammonia–ammonium chloride solution. Although amino acids or peptides containing –SH or –S–S– radicals produced a polarographic catalytic wave, methionine, djenkolic acid and S-benzylcysteine did not.[4] While Brdicka[5] has proposed a mechanism for the production of the double wave some points still remain unexplained.

Brdicka[6] found that the catalytic waves obtained with serum from cancer patients in general were smaller than those observed with normal sera. The difference was more pronounced if the protein was denatured. However, it was found[7] that after treating serum with a deproteinizing agent (sulfosalicylic acid), catalytic polarographic activity observed in the filtrate was generally higher in sera from cancer patients.

In the present studies the polarographic behavior of the three octapeptide hormones from the posterior pituitary gland has been investigated as a model to learn more of the nature of the protein double wave. All three of these polypeptides, lysine-vasopressin, arginine-vasopressin and oxytocin, may be obtained in highly purified form and their structures are established.[8] These peptides produced polarographic catalytic waves in both cobaltous and cobaltic ammonia–ammonium chloride solutions. To simplify presentation the data have been divided into two parts. The present paper describes the behavior in cobaltous ammonia–ammonium chloride solutions and the following paper describes the behavior in cobaltic ammonia–ammonium chloride solutions.

### Experimental

**Hormone Purification.**—Oxytocin was prepared according to the method of Livermore and du Vigneaud[9] and had a potency of 380 units per mg. by the chicken blood pressure method.[10]

Arginine-vasopressin and lysine-vasopressin were prepared by the method of Ward and Guillemin[11] and had a potency of 380 and 260 units pressor activity per mg., respectively. All chemicals used were C.P. or analytical reagent.

**Preparation of Bis-(acetyl)-lysine-vasopressin.**—The acetylation of lysine-vasopressin was carried out with acetic anhydride (15 mg. lysine-vasopressin in 1 ml. of water, 10 $\mu$l. of acetic anhydride and 15 $\mu$l. triethylamine, reacted 30 minutes in an ice-bath). Unreacted lysine-vasopressin was removed by countercurrent distribution in the system 0.1% acetic acid–*sec*-butyl alcohol. Vasopressin has a distribution coefficient of 0.15 in this system. The product of the acetylation had a distribution coefficient of 0.37. The acetylated material was put through the distribution twice, 45 transfers each time, to insure complete removal of vasopressin as shown in Fig. 1A and B; final yield, 9 mg. The ninhydrin reaction with 250 $\mu$g. of this material gave an O.D. of 0.060 while 250 $\mu$g. of lysine-vasopressin under the same

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(2) R. Brdicka, *Collection Czechoslov. Chem. Commun.*, **5**, 112 (1937).

(3) R. Brdicka, *ibid.*, **5**, 148 (1937).

(4) E. R. Smith and C. J. Rodden, *Bur. Standards J. Research*, **22**, 146 (1939).

(5) R. Brdicka, *Collection Czechoslov. Chem. Commun.*, **11**, 614 (1939).

(6) R. Brdicka, *Nature*, **139**, 330, 1020 (1937); *Acta Unio Intern. Contra Cancrum*, **3**, 13 (1938); *Compt. rend. Soc. Biol.*, **128**, 54 (1938).

(7) R. Brdicka, *Klin. Wochschr.*, **18**, 305 (1939).

(8) V. du Vigneaud, C. Ressler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953); V. du Vigneaud, H. C. Lawler and E. A. Popenoe, THIS JOURNAL, **75**, 4880 (1953).

(9) A. H. Livermore and V. du Vigneaud, *J. Biol. Chem.*, **180**, 365 (1949).

(10) We are indebted to Dr. R. Guillemin, Baylor University Medical College, Houston, Texas, for the bioassays of oxytocin and vasopressin.

(11) D. N. Ward and R. Guillemin, *Proc. Soc. Exptl. Biol. Med.*, **96**, 568 (1957).

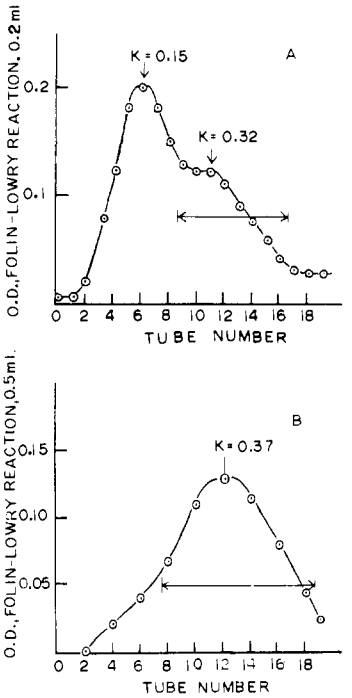


Fig. 1.—(A) Counter-current distribution curve of the original acetylation product from 15 mg. lysine-vasopressin treated with acetic anhydride: system, 0.1% acetic acid–sec. butyl alcohol; 45 transfers; the arrow indicates the tubes pooled for re-distribution. (B) Re-distribution of acetylated product obtained in (A) solvent system and number of transfers are the same.

conditions gave an O.D. of 0.548, indicating both amino groups have been acetylated. The phenolic hydroxyl group on tyrosine was not acetylated under these conditions since a shift in ultraviolet absorption spectrum was obtained, as with natural lysine-vasopressin, measured in acid and base. The maxima in 0.1 *N* HCl were at 270 mμ., while in 0.1 *N* NaOH the maxima were at 286 mμ (solutions: 1 mg./3 ml.) Crammer and Neuberger[12] have shown this shift to be due to the ionization of the phenolic hydroxyl group. The acetylated material had a potency of less than 1 unit of pressor activity per mg.

**Preparation of S,S′-Bis-(benzyl)-lysine-vasopressin.**—The benzylation of vasopressin was carried out by reducing 64 mg. of lysine-vasopressin with sodium in liquid ammonia until the sodium blue color persisted for 30 sec., and then reaction with 20 mg. of benzyl chloride as described by Sifferd and du Vigneaud for the preparation of S-benzylcysteine.[13] The resulting S,S′-bis-(benzyl)-lysine-vasopressin, after removal of ammonia, was isolated by countercurrent distribution in the system 0.1% acetic acid, *sec*-butyl alcohol. The *K* value for the major component was 0.33 after 110 transfers and showed a curve slightly narrower than that predicted by the theoretical curve (Fig. 2) for a substance with this partition coefficient;[14] final yield (tubes 19–35, Fig. 2), 54 mg. This material had a pressor activity of less than 1 unit per mg. Debenzylation studies with sodium and liquid ammonia have demonstrated the regeneration of up to 50% of the theoretical pressor activity.

**Preparation of Serum Filtrate for Polarography.**—Serum which had been stored in the cold for short periods of time was precipitated with 3% sulfosalicylic acid, filtered and the filtrate dialyzed in the cold in order to remove excess sulfosalicylic acid. This is the method of Brdicka[7] modified to include the dialysis which gives a product with slightly higher well-defined polarographic waves. The exact nature of this material has not been established, but it is presumably a mixture of rather high molecular weight polypeptides.

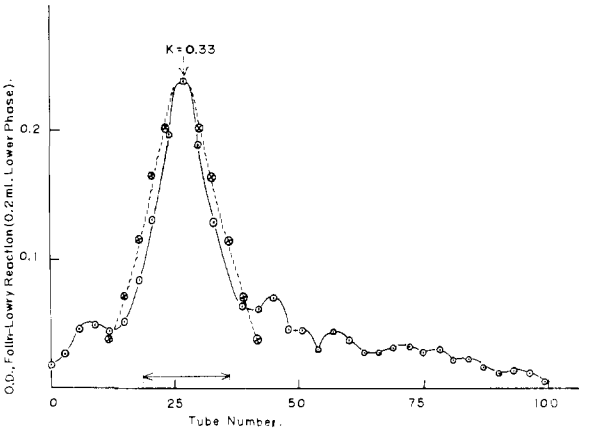


Fig. 2.—Counter-current distribution curve for the product of the benzylation of reduced lysine-vasopressin (64 mg.) in the system 0.1% acetic acid–*sec*-butyl alcohol; 110 transfers; the arrow indicates those tubes from which the benzylated product (54 mg.) was obtained.

**Polarography.**—A Sargent-Heyrovsky Polarograph, Model XII, was used for this study. The galvanometer calibration factor was $5.8 \times 10^{-9}$ amp./mm. with the capillary immersed in distilled water with a mercury pressure head of 49 cm., $m = 2.18$ mg./sec., $t = 3.94$ sec. The radius of the orifice was calculated by Muller's method[15] as 31.03 μ. All polarograms were recorded at sample temperature of 25.0 ± 0.2°. All polarograms were run with a very low concentration of Triton 100X to suppress the cobalt maximum and show better definition of the catalytic waves. At this low concentration of suppressor the height of the catalytic waves is virtually unaffected. Nitrogen gas was used to remove dissolved oxygen from the electrolyte before running the polarograms. All *p*H values were measured with a Beckman Model H-2 *p*H-Meter after completion of the polarogram. The potentials were recorded at the dropping mercury electrode against a saturated calomel electrode (s.c.e.) connected by a salt bridge having a low resistance (750 ohm.)

In this paper and the one following, $i_{p1}$, $i_{p2}$, $i_{p3}$ and $i_{p4}$ refer to the magnitudes of wave I, II, III and IV, respectively, measured from the means of the oscillations on the tracing from the level of the cobalt limiting current up to the peaks of the individual waves. The peak point of the maximum waves was used as peak potential *vs.* s.c.e., and these are referred to as $E_{p1}$, $E_{p2}$, $E_{p3}$ and $E_{p4}$.

## Results and Discussion

**1. Origin of the Polarographic Reduction Waves of Oxytocin, Lysine- and Arginine-vasopressin.**—Cystine showed a small maximum wave[16] in cobaltous ammonia–ammonium chloride solutions as Fig. 3A but not with cobaltic ion except at concentrations much higher than presented here. All three peptides, oxytocin (OXY), lysine-vasopressin (LVP) and arginine-vasopressin (AVP) showed waves (I and II, Fig. 3B, C and D) with maxima, plus one (IV, Fig. 3B) or two (III and IV, Fig. 3C and D) waves without a maximum at a more negative potential. Oxytocin showed an additional wave with a maximum (III, Fig. 3B). The peptides gave similar patterns in the presence of either cobaltic or cobaltous ions. In general, wave height of the

(12) J. L. Crammer and A. Neuberger, *Biochem. J.*, **37**, 302 (1943).

(13) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(14) D. N. Ward wishes to acknowledge an earlier study in collaboration with Prof. V. du Vigneaud at Cornell University Medical College during which this compound was prepared. The *K* value in the same solvent was 0.36 in that study, which has not been published.

(15) O. H. Muller, THIS JOURNAL, **66**, 1019 (1944).

(16) Recently, we have found another wave for cystine at more negative potential, under these conditions, which is currently being investigated.

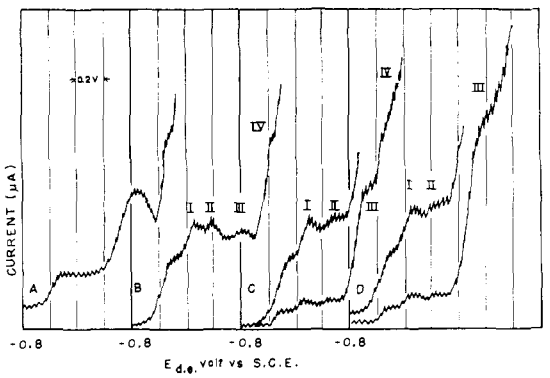


Fig. 3.—Polarograms of cystine (A), oxytocin (B), lysine-vasopressin (C) and arginine-vasopressin (D) in cobaltous ammonia–ammonium chloride solution: cystine, 5.5 μg./ml., Sen = $^1/_{100}$; oxytocin, 4.6 μg./ml., Sen = $^1/_{50}$; lysine-vasopressin, 5.0 μg./ml., Sen = $^1/_{50}$; arginine-vasopressin, 5.0 μg./ml., Sen = $^1/_{50}$ or $^1/_{200}$; supporting electrolyte: cobaltous chloride 1.1 m$M$/l., $NH_4Cl$ 0.1 $M$ and $NH_4OH$ 0.1 $M$ containing 2 drops of Triton X100 0.1% solution per 5 ml. of sample volume.

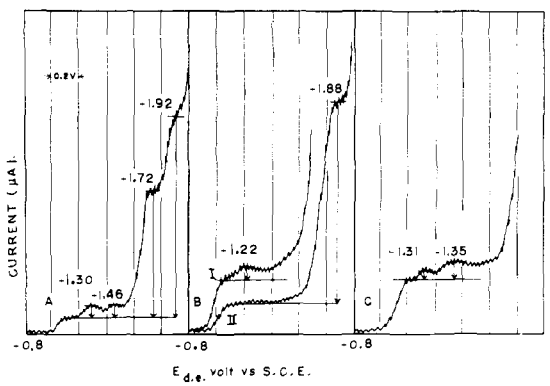


Fig. 4.—Polarograms of lysine-vasopressin, S,S′-bis-(benzyl)-lysine-vasopressin and bis-(acetyl)-lysine-vasopressin in cobaltous ammonia–ammonium chloride solution: A, lysine-vasopressin: 5.0 μg./ml., Sen = $^1/_{500}$; B, S,S′-bis-(benzyl)-lysine-vasopressin: (I) 30 μg/ml., Sen = $^1/_{50}$, (II) 20 μg./ml., Sen = $^1/_{200}$; C, bis-(acetyl)-lysine-vasopressin: 10.0 μg./ml., Sen = $^1/_{50}$; supporting electrolyte: cobaltous chloride 1.1 m$M$/l., $NH_4Cl$, 0.1 $M$ and $NH_4OH$, 0.1 $M$ containing 2 drops of Triton X100 0.1% solution per 5 ml. of sample volume.

peptides in cobaltous ammonia–ammonium chloride solution was smaller than in cobaltic ammonia–ammonium chloride solution. The similarity of the polarogram for lysine- and arginine-vasopressin is especially noteworthy in view of the close similarity of structure.[8] Millar[17] observed a polarographic wave at approximately −1.7 or −1.8 v. with bovine plasma albumin, insulin, etc., under similar conditions, which would correspond to wave III in Fig. 3. After acetylation this wave was suppressed, so he concluded this was associated with the free amino radical. In Fig. 4, the polarograms of vasopressin, acetylated lysine-vasopressin, and S,S′-bis-(benzyl)-lysine-vasopressin are shown. Wave III of acetylated lysine-vasopressin (Fig. 4C) is completely suppressed at the same peptide concentration. At six times this concentration of peptide (30 μg./ml.) a slight indication of wave III was obtained (2.5 μA. compared to 40 μA. in the 5 μg./ml. solution of vasopressin). According to Millar's explanation[17] for wave III of insulin, free amino groups affect wave III through their influence on the adsorption process and also because they are involved in the electrode reaction which produces the wave III current. Millar suggested the free amino groups of the protein enhance the tendency of the protein to be adsorbed at the electrode surface, followed by a reduction of the protonated amino groups, and this electrolytic reduction of hydrogen supplied by charged amino groups provides the current of wave III. As will be described below, the height of wave III of lysine-vasopressin and arginine-vasopressin decreases suddenly with increasing concentration of ammonia. In alkaline media the concentration of $RNH^+_3$ groups decreases. Thus, our other data also support the mechanism proposed by Millar.

In order to learn more concerning the nature of waves I and II in the polarographic pattern, benzylated vasopressin was studied. As can be seen from Fig. 4, the bis-benzyl derivative gave a polarographic pattern which was especially useful in establishing the nature of the groups giving rise to waves I, II and III. Wave II was completely abolished at all concentrations studied (up to 30 μg./ml.), thus this wave requires the presence of the disulfide bond for its generation. Wave I was detectable at a concentration of 30 μg./ml. but was markedly suppressed in magnitude as compared with the original vasopressin. Wave III was virtually eliminated by benzylation, even at the 30 μg./ml. level. Thus, in addition to a free amino group there must be a disulfide bond present in order to generate wave III.

To summarize for vasopressin, the source of wave I has not been determined in our studies, but from the pattern of the S,S′-bis-(benzyl)-lysine-vasopressin it may involve the disulfide bond, at least indirectly, since the presence of this bond greatly enhances the amplitude of wave I (approximately 25-fold). Furthermore, wave I shows no dependence on ammonia concentration in the bis-benzyl derivative, but in the presence of the disulfide bond it does exhibit such a concentration dependence which parallels that of wave II (see Section 4). Wave II is obtained only in the presence of a disulfide bond and basic amino groups in the molecule. Wave IV requires only basic groups in the molecule for its production.

The source of waves I and II for oxytocin in cobaltous chloride is not clear, since the character of these waves does not parallel their counterpart in the vasopressin pattern with respect to the effect of ammonia concentration (see below). It is possible that the difference may be related to the net charge on the peptide at the $p$H of the solutions used for polarography ($p$H = 9.6 to 10.0 under these conditions). The net charge for vasopressin would be approximately +1 while that of oxytocin would be near zero.

**2. The Relationship of Wave Height to Peptide Concentration.**—Wave height as a function of cys-

(17) G. J. Millar, *Biochem. J.*, **53**, 385 (1953).

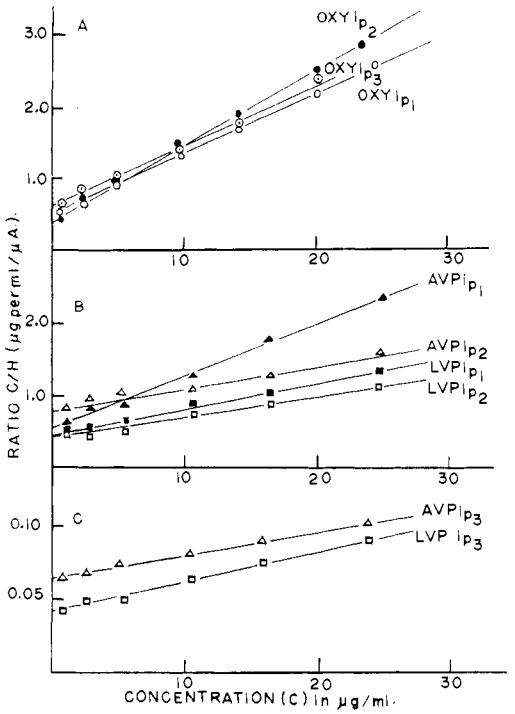


Fig. 5.—The relation between C/H and C test for conformity to the Langmuir adsorption isotherm: A, oxytocin; B and C, lysine- and arginine-vasopressin, respectively; supporting electrolyte, cobaltous chloride 1.1 m$M$/l., $NH_4Cl$ 0.1 $M$ and $NH_4OH$ 0.1 $M$ containing 2 drops of Triton X100 0.1% solution per 5 ml. of sample volume.

tine concentration was found by Brdicka[18] to resemble a Langmuir adsorption isotherm. Millar[17] found a similar relationship for a number of purified proteins. In the present study all three peptides showed a relationship of wave height to concentration that also resembled a Langmuir adsorption isotherm. Langmuir's adsorption isotherm equation may be rearranged to give a straight line plot

$$C/H = 1/W \cdot Z + C/Z$$

assuming $N$ in the original equation (number of molecules adsorbed on the surface) corresponds to the wave height ($H$, in μA). "$Z$" is a constant proportional to the area on the surface occupied by adsorbed molecules, "$W$" is the adsorption coefficient and "$C$" is the concentration expressed as μg/ml. The plot of $C/H$ against $C$ (Fig. 5) was a straight line showing conformity to the Langmuir type of equation in all instances except wave IV for oxytocin. In this instance a plot of wave height *versus* concentration gave a straight line curve. Current for wave IV for the vasopressin was too high for accurate measurement over most of the concentration range studied. Thus, one may conclude that the polarographic reduction waves I, II and III produced by oxytocin and vasopressin in cobaltous ammonia–ammonium chloride solution result from an adsorption reaction at the dropping mercury electrode like the cystine wave or protein double wave. The values for $Z$ and $W$ calculated from the slope and intercept values in Fig. 5 are listed in Table I.

(18) R. Brdicka, *Biochem. Z.*, **272**, 104 (1934).

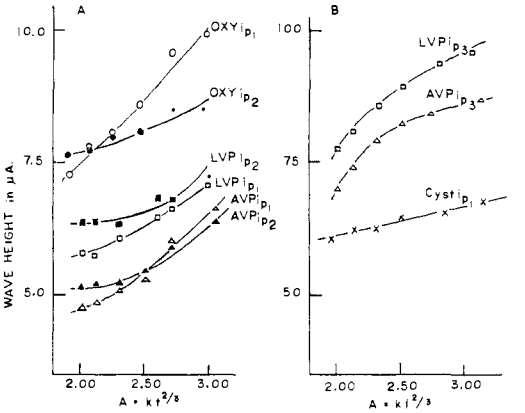


Fig. 6.—(A) The relationship between the wave height and $t^{2/3}$ as a factor of surface area of dropping mercury with changing effective pressure on the dropping mercury: OXY $i_{p1}$ and OXY $i_{p2}$ = oxytocin, waves I and II; AVP $i_{p1}$ and AVP $i_{p2}$ = arginine-vasopressin, waves I and II; LVP $i_{p1}$ and LVP $i_{p2}$ = lysine-vasopressin, waves I and II. (B) CYST $i_{p1}$ = cystine, wave I; AVP $i_{p3}$ = arginine-vasopressin, wave III; LVP $i_{p3}$ = lysine-vasopressin, wave III; supporting electrolyte, cobaltous chloride 1.1 m$M$/l., $NH_4Cl$, 0.1 $M$ and $NH_4OH$, 0.1 $M$ containing 2 drops of Triton X100 0.1% solution per 5 ml. of sample volume. Oxytocin, 13.9 μg./ml.; arginine-vasopressin, 5.0 μg./ml.; lysine-vasopressin, 5.0 μg./ml.; cystine, 5.5 μg./ml.

**3. The Relation of Wave Height to Pressure on the Dropping Mercury Electrode.**—In the Ilkovic equation[19] the diffusion current $i_d$ is very nearly directly proportional to the product $m^{2/3}t^{1/6}$ where "$m$" is the weight of mercury flowing from the capillary per second and "$t$" is the drop time in seconds. It is evident that "$m$" and "$t$"

TABLE I

VALUES FOR $Z$ AND $W$ CALCULATED FROM FIGURE 5

| Peptide | | $W$ | $Z$ |
|---|---|---|---|
| Oxytocin | OXY $i_{p1}$ | 0.163 | 41.6 |
| | OXY $i_{p2}$ | .272 | 33.0 |
| | OXY $i_{p3}$ | .127 | 43.0 |
| Arginine-vasopressin | AVP $i_{p1}$ | .12 | 50 |
| | AVP $i_{p2}$ | .045 | 100 |
| | AVP $i_{p3}$ | .022 | 238 |
| Lysine-vasopressin | LVP $i_{p1}$ | .076 | 100 |
| | LVP $i_{p2}$ | .083 | 100 |
| | LVP $i_{p3}$ | .044 | 181.8 |

will depend on the dimensions of the dropping electrode capillary and on the pressure on the dropping mercury. But the Ilkovic equation is not applicable to catalytic currents of the type observed in the case of cystine and the peptides or proteins mentioned above. As noted, Brdicka was able to derive an equation, based on Langmuir's adsorption isotherm, which expressed fairly well the relationship between catalytic current and concentration, but this equation assumes a constant value for "$m$" and "$t$." There are few data in the literature concerning the effect of pressure on the dropping mercury and the height of catalytic waves. Therefore, the effect of mercury pressure

(19) D. Ilkovic, *Collection Czechoslov. Chem. Communn.*, **6**, 498 (1934).

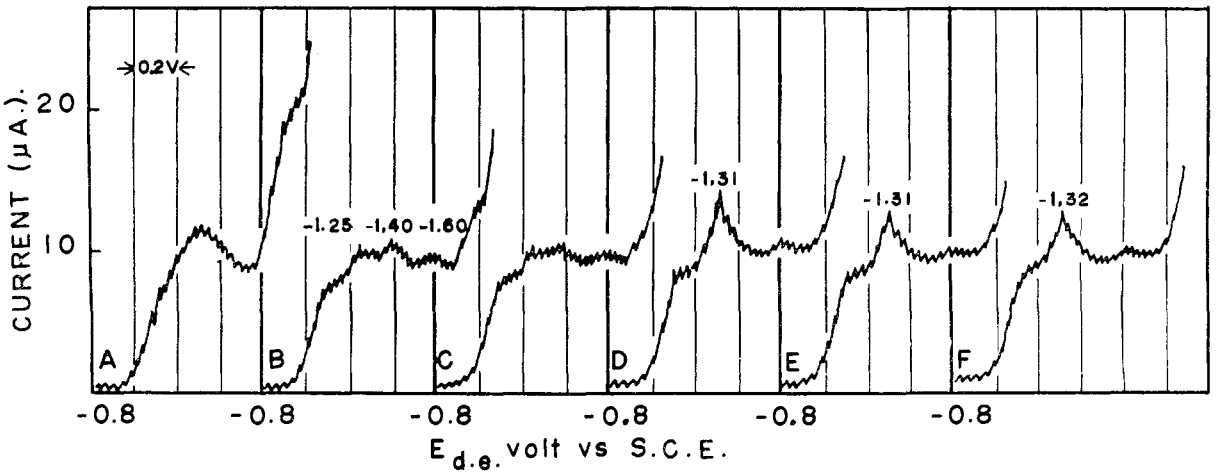


Fig. 7.—The effect on the oxytocin catalytic wave of alteration of the concentration of ammonia: A, 0; B, 0.05 *M*; C, 0.1 *M*; D, 0.4 *M*; E, 0.6 *M*; F, 1.0 *M* of ammonia concentration. Oxytocin, 3.0 μg./ml. in cobaltous chloride 1.1 m*M*/l., $NH_4Cl$ 0.1 *M* containing 2 drops of Triton X100 0.1% solution per 5 ml. of sample volume.

on the wave height for oxytocin and vasopressin was investigated. The relation of the wave height of cystine, oxytocin, lysine- and arginine-vasopressin to the effective pressure ($P = \sqrt{h_{corr.}}$) on the dropping mercury, calculated by Kucera's method[20] was studied. The wave height decreased with increasing mercury pressure. The $i_{p2}$ of oxytocin and $i_{p1}$, $i_{p2}$ of lysine- and arginine-vasopressin and $i_{p1}$ of cystine showed the same behavior, that is, a decreasing current up to $\sqrt{h_{corr.}} = 8.0$, at which point a limiting value was reached and the wave height then remained constant. The $i_{p3}$ of lysine- and arginine-vasopressin and $i_{p1}$ of oxytocin were linear in their response and showed a constant decrease up to the highest pressure studied.

Since the relation between wave height and the concentration of peptide was related to an adsorption at the dropping mercury electrode, the wave height was also studied with respect to the change of surface area of the dropping mercury as related to the mercury pressure. Since "*m*" is increasing and "*t*" decreases with increasing mercury pressure, the maximum surface of the mercury will also decrease with increasing mercury pressure. The maximum surface of the mercury drop will also decrease according to the equation $A = kt^{2/3}$ where "*t*" is the drop time, "*A*" the maximum surface area and "*k*" a constant.[21] The relationship between wave height and maximum surface area of the mercury drop is shown in Fig. 6. The wave height decreased with decreasing surface area of the dropping mercury as would be expected if the reduction of the peptide at the dropping mercury electrode involved an adsorption phenomenon.

**4. The Influence of Ammonia Concentration on the Polarographic Pattern.**—The reactions which are responsible for the catalytic waves of cystine, peptides and proteins are not well understood, but cobalt and ammonia are necessary constituents of the electrolyte and a cobalt–ammonia complex must be an essential component of this system. The role of ammonia in the process was studied.

Brdicka[3] observed that the cystine wave was increased with increasing concentration of ammonia. The protein double wave also showed the same behavior. In Fig. 7, the changes in the general character of the polarographic pattern of oxytocin are shown over the concentration of ammonia from 0 to 1.0 *N*. Without ammonia a round maximum wave at −1.35 volts and another wave at −1.75 volts were present. With increasing concentration of ammonia, waves I, II, III and IV appear. At higher concentrations $i_{p1}$ and $i_{p2}$ seem to become one peak while $i_{p3}$ and $i_{p4}$ are depressed. Wave $i_{p4}$ eventually disappears at the higher concentration.

In Fig. 8, the effect on the arginine-vasopressin polarographic pattern with various concentrations of ammonia is shown. In the absence of ammonia

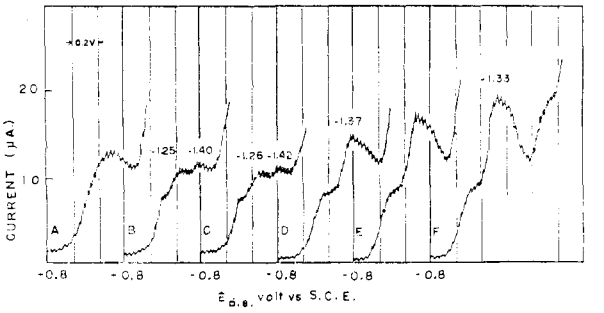


Fig. 8.—The effect on the arginine-vasopressin catalytic wave of alteration of the concentration of ammonia: A, 0; B, 0.05 *M*; C, 0.1 *M*; D, 0.4 *M*; E, 0.6 *M*; F, 1.0 *M* of ammonia concentration. Arginine-vasopressin, 5.0 μg./ml. in cobaltous chloride 1.1 m*M*/l., $NH_4Cl$ 0.1 *M* containing 2 drops of Triton X100 0.1% solution per 5 ml. of sample volume.

the pattern was very similar to that of oxytocin, but with increasing ammonia concentration more marked changes appeared. The $i_{p1}$ becomes higher than $i_{p2}$, then above 0.4 *N* ammonia $i_{p1}$ and $i_{p2}$ seemed to become one rounded peak and $i_{p3}$ was depressed. The merging of wave I and II results

(20) G. Kucera, *Ann. physik.*, **11**, 529 (1903).

(21) "Textbook of Theoretical Polarography," published by Committee on Polarography in Japan, 1956.

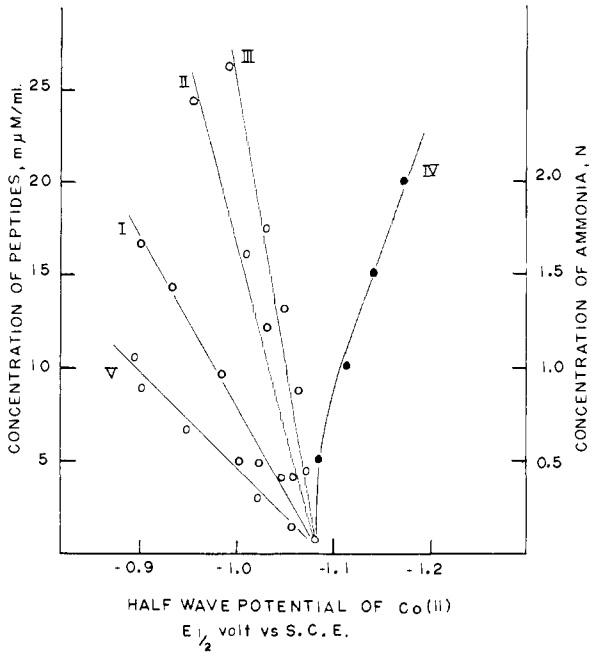


Fig. 9.—The relation between half wave potential of Co(II) and peptide concentration or ammonia concentration: I, lysine-vasopressin; II, S,S′-bis-(benzyl)-lysine-vasopressin; III, bis-(acetyl)-lysine-vasopressin; IV, half wave potential of Co(II) with different concentrations of ammonia (right hand scale); V, cystine. Supporting electrolyte: cobaltous chloride 1.1 m*M*/l., $NH_4Cl$ 0.1 *M* and $NH_4OH$ 0.1 *M* containing 2 drops of Triton X100 0.1% solution per 5 ml. of sample volume (except curve IV).

mainly from a shift of wave I to a more negative potential, although wave II shifts slightly toward a more positive potential. Lysine-vasopressin showed the same behavior with varying ammonia concentrations as described for arginine-vasopressin. The tendency of peaks I and II to merge with increasing ammonia concentration has been reported by Brdicka for the protein double wave.[22] Here the character of the polarographic pattern of proteins more closely resembles that of vasopressin. Qualitatively the same effect was obtained with oxytocin (Fig. 7), but the magnitude of the current was much less.

The variation of wave height with change in ammonia concentration for both vasopressins was very similar to that described by Millar[23] for bovine plasma albumin. Waves I and II increased with increasing ammonia concentration, while wave III decreased; the wave height was not a linear function of ammonia concentration in either case.

**5. The Effect of Peptide Concentration on Half-Wave Potential of Cobaltous Ion.**—With increasing concentration of ammonia the extent of complex formation with cobalt is increased and concomitantly its half-wave potential is shifted more negative.[24] It was reasoned that the peptide would reverse such a process provided the peptide–cobalt complexes were more stable than those with ammonia (since the ammonia is present in a far greater excess). At the outset one cannot anticipate the nature of any shift in half-wave potential since the peptide–cobalt complex itself may have an either greater or lesser effect than the ammonia in causing a negative shift. Accordingly, the cobalt half-wave potential was studied with increasing concentrations of peptide, using the three peptides, lysine-vasopressin, S,S′-bis-(benzyl)-lysine-vasopressin and bis-(acetyl)-lysine-vasopressin and a constant concentration of ammonia. For comparison the effect of cystine was also studied (Fig. 9V). The effect of increasing concentrations of ammonia on the cobaltous ion half-wave potential is also shown (Fig. 9IV).

It can be seen that cystine shows the greatest effect on the half-wave potential and in all probability is more effective in competing with the ammonia for formation of a cobalt complex, although other interpretations may be possible. If such an assumption is valid, then from Fig. 9 it may be concluded that lysine-vasopressin forms a cobalt complex almost as stable as that of cystine, the S,S′-bis-(benzyl)-lysine-vasopressin and bis-(acetyl)-lysine-vasopressin form a less stable complex with cobaltous ion.

(22) R. Brdicka, *Research*, **1**, 25 (1947).

(23) G. J. Millar, *Biochem. J.*, **53**, 393 (1953).

(24) H. A. Laitinen, J. C. Bailar, Jr., H. F. Holtzclaw, Jr., THIS JOURNAL, **70**, 2999 (1948); H. A. Laitinen, A. J. Frank and P. Kivalo, *ibid.*, **75**, 2865 (1953); H. A. Laitinen and P. Kivalo, *ibid.*, **75**, 2198 (1953).