D_2O; subsequently the tube was heated at 100°; the positions and ratios of peak heights remained unchanged. In all these experiments, the peaks corresponding to the aromatic hydrogen atoms, to the methylene hydrogen atoms and to the methyl hydrogen atoms were present in approximately the theoretical ratios of 1:2:3, and since the peaks were unchanged with time, the measurements showed that there was little or no exchange with the solvent at the methylene group or elsewhere in the pyrimidine ring, under the experimental conditions employed. However, in most experiments, neither the heights nor the areas under the peaks were in exactly the theoretical ratios; a typical example of peak heights was 1:1.8:3.0, although some others corresponded more nearly to 0.8:2.0:3.0, and one set was exactly 1.0:2.0:3.0. The variations depended to some extent on the precise balancing of the N-M-R Spectrometer. Similar quantitative deviations were obtained with other compounds (*e.g.*, ethanol). Since the ratios of the peaks in the sodium salt of II are strictly independent of time, the possibility of exchange in the salt under the conditions of the experiments is eliminated.

Manometric Measurements.—The decarboxylation was carried out at 50° as in the experiments to measure deuterium exchange. After measured time intervals, the solutions were cooled, and one ml. introduced into a standard Warburg flask; 1 ml. of 2 *N* HCl was introduced into the side arm. When the flask had come to temperature in the thermostat for the Warburg, the solutions were mixed, and the evolution of CO_2 noted. The method was standardized with solutions of K_2CO_3, and all the Warburg flasks calibrated. A solution of 326 mg. of thiamin chloride hydrochloride and 105 mg. of sodium pyruvate in 10 ml. of 0.196 *N* NaOH gave a 40% yield of CO_2 in six hours; a solution of 330 mg. of thiamin chloride hydrochloride and 1.07 g. of sodium pyruvate in 10 ml. of 0.196 *N* NaOH gave 135% yield of CO_2 based on the thiamin present. These manometric experiments were refined and confirmed by Dr. DeLos F. DeTar, who has observed even larger yields of CO_2 based on thiamin introduced.

Acknowledgment.—The authors wish to thank the National Science Foundation and the Guggenheim Foundation for their generous support of this work.

CAMBRIDGE 38, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, WEIZMANN INSTITUTE OF SCIENCE]

Poly-L-histidine

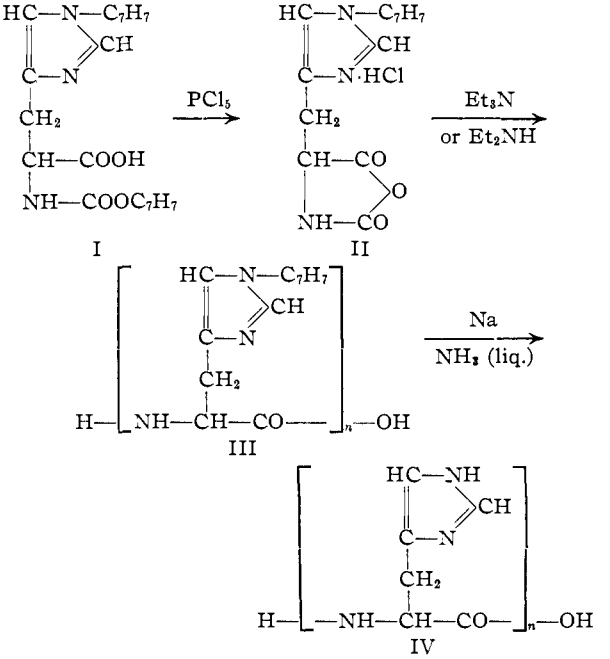
BY ABRAHAM PATCHORNIK, ARIEH BERGER AND EPHRAIM KATCHALSKI

RECEIVED MAY 25, 1957

Poly-L-histidine (IV) was synthesized by the polymerization of 1-benzyl-N-carboxy-L-histidine anhydride (II) in dioxane, and reduction of the resulting poly-1-benzyl-L-histidine (III) with sodium in liquid ammonia. The potentiometric titration of a poly-L-histidine preparation with an average degree of polymerization $n = 15$ could be described by equation 1 assuming an intrinsic dissociation constant, pK_0 6.15, for the imidazolium groups of the polymer. The apparent heat of ionization of the imidazole groups of poly-L-histidine, $\Delta H' = 5.2$ kcal./mole residue, was calculated by means of equation 3. Poly-L-histidine formed insoluble complexes with copper, zinc, magnesium, cobalt, silver and mercury. Poly-1-benzyl-L-histidine formed sparingly soluble salts with many mineral acids.

The imidazole groups of the histidine residues are responsible for most of the buffering capacity of proteins in the physiological *p*H range.[1] They are also capable of combining with various metal ions and appear to constitute the principal sites for metal binding in proteins.[2] Poly-L-histidine was synthesized to provide a model compound for the investigation of the properties of the histidine residue in high molecular weight compounds.

In the synthesis of poly-L-histidine, outlined in the following scheme, the imidazole imino group was reversibly blocked by benzylation. The starting monomer, 1-benzyl-N-carboxy-L-histidine anhydride hydrochloride (II), was prepared from 1-benzyl-N-carbobenzoxy-L-histidine (I)[3] by treatment with phosphorus pentachloride. A solution of the free 1-benzyl-N-carboxyl-L-histidine anhydride in dioxane was obtained by neutralizing II with one mole of triethylamine and removing the insoluble triethylammonium chloride. In this solution polymerization was initiated at room temperature either by diethylamine[4,5] or triethylamine.[6] The poly-1-benzyl-L-histidine (III) which separated out was treated with sodium in liquid ammonia to remove the protecting benzyl groups.[7]



For purification the resulting poly-L-histidine (IV) was dissolved in dilute acid and precipitated with dilute alkali.

(1) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943.

(2) C. Tanford, THIS JOURNAL, **74**, 211 (1952); F. R. N. Gurd and D. S. Goodman, *ibid.*, **74**, 670 (1952); J. T. Edsall, G. Felsenfeld, D. S. Goodman and F. R. N. Gurd, *ibid.*, **76**, 3054 (1954).

(3) B. G. Overell and V. Petrow, *J. Chem. Soc.*, 232 (1955).

(4) *Cf.* S. G. Waley and J. Watson, *Proc. Roy. Soc.* (*London*), **A199**, 499 (1949).

(5) M. Sela and A. Berger, THIS JOURNAL, **77**, 1893 (1955).

(6) E. R. Blout and R. H. Karlson, *ibid.*, **78**, 941 (1956); D. G. H. Ballard and C. H. Bamford, *J. Chem. Soc.*, 381 (1956).

(7) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).

The crystalline 1-benzyl-N-carboxyl-L-histidine anhydride hydrochloride (II) is stable at room temperature when kept dry but decomposes to yield 1-benzyl-L-histidine and carbon dioxide in the presence of mineral acids.

Poly-1-benzyl-L-histidine (III) yielded upon acid hydrolysis 1-benzyl-L-histidine quantitatively and had the expected neutralization equivalent as determined by anhydrous titration with perchloric acid. Preparations of III had an average degree of polymerization n = 15 to 50 as calculated from end-group analysis.[5] Poly-1-benzyl-L-histidine hydrochloride is soluble in 0.1 N hydrochloric acid but is precipitated at higher chloride concentrations. An aqueous dilute solution of the hydrochloride gives sparingly soluble salts with many anions. The precipitates contain approximately one equivalent of the respective acid per benzylhistidine residue.

Poly-L-histidine (IV) as prepared contained half a molecule of water per histidine residue which could not be removed by drying *in vacuo* (0.1 mm.) at 100°; however, a water-free picrate could be prepared. A chromatogram of an acid hydrolysate of poly-L-histidine revealed only one spot with ninhydrin having the same R_f as L-histidine. The amino nitrogen content and the optical rotation of the hydrolysate corresponded to a quantitative yield of L-histidine.

The solubility of poly-L-histidine in dilute acids (below pH 5.8) and in strong alkali (above 3.5 N NaOH) shows that IV behaves as a polyampholyte. This is consistent with the amphoteric nature of the imidazole group which has been demonstrated by Wieland and Schneider[8] who assigned acidic dissociation constants of about pK 7.0 and 13 to the imidazolium ion and the imino group of the uncharged imidazole, respectively. The absence of free imino groups in polybenzylhistidine would therefore explain its insolubility in strong alkali.

The potentiometric titration of poly-L-histidine (n average 15) in a solution of an ionic strength of 0.1, and the variation of the degree of ionization, α, with ionic strength at pH 5.75 are given in Figs. 1 and 2, respectively. The drawn curve in Fig. 1

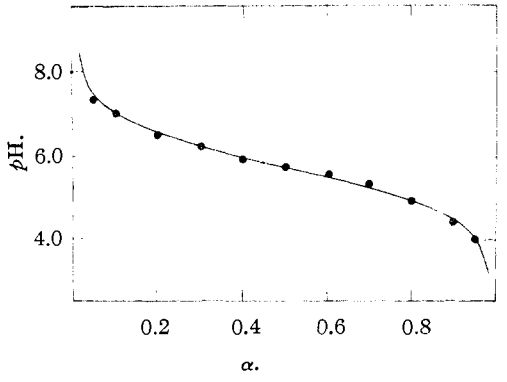


Fig. 1.—Potentiometric titration of poly-L-histidine (n average 15) at 25°, and an ionic strength of 0.1 (NaCl). The full curve was computed from equation 1; concentration of polymer 25 mg. per ml.

(8) T. Wieland and G. Schneider, *Ann.*, **580**, 159 (1953).

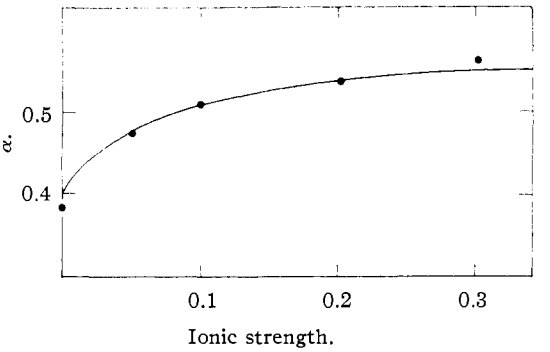


Fig. 2.—Dependence of the degree of ionization, α, of poly-L-histidine (n average 15) at 25°, and at pH 5.75 on the ionic strength. The full curve was computed from equations 1 and 2.

is a plot of equation 1 assuming an intrinsic dis-

$$pH = pK_0 + \log \frac{1-\alpha}{\alpha} - 0.868\, n\alpha w \qquad (1)$$

sociation constant of pK_0 6.15, and an electrostatic interaction factor w = 0.0575. This equation has been used successfully to describe the potentiometric titration of proteins.[9,10] The drawn curve in Fig. 2 is a plot of the degree of ionization, α, as a function of ionic strength at pH 5.75 as calculated from (1) and (2) assuming a spherical molecule with a radius b = 21 Å. and a distance of closest approach a = 23 Å. D is the dielectric constant of

$$w = \frac{e^2}{2DkT}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) \qquad (2)$$

water, k the Boltzmann constant, T the absolute temperature, e the electronic charge and κ has its usual significance in the Debye theory.

The intrinsic dissociation constant assumed (pK_0 6.15) is within the range (pK 5.6 to 7.0) reported for the acidic dissociation constant of the histidine residue in proteins[11] and approximates that of the imidazole group of histidine (pK_0 6.00).[12]

The temperature dependence of the pH at an ionization of α = 0.65, in the range of 10 to 40° of a poly-L-histidine (n = 15) solution is given in Fig. 3. From the slope of the curve the apparent heat of ionization of the imidazole groups in polyhistidine, $\Delta H'$ = 5.2 kcal./mole residue, was calculated by means of eq. 3. A value of 6.5 kcal./mole has been reported for the imidazole residues of serum albumin.[13]

$$\Delta H' = 2.303R\left[\frac{\partial pH}{\partial(1/T)}\right]_{\alpha=0.65} \qquad (3)$$

Preliminary experiments have shown that polyhistidine forms insoluble complexes with copper, zinc, magnesium, cobalt, silver and mercury. With the exception of the latter they dissolve in dilute acid. When a suspension of polyhistidine at pH 9.7 was titrated with silver nitrate the pH dropped gradually to a value of 5.4. Figure 4 gives both the change in pH and the change of the silver elec-

(9) R. K. Cannan, A. Kibrick and A. H. Palmer, *Ann. N. Y. Acad. Sci.*, **41**, 243 (1941); R. K. Cannan, *Chem. Revs.*, **30**, 395 (1942).
(10) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).
(11) See ref. 1, p. 445; C. Tanford, THIS JOURNAL, **72**, 441 (1950).
(12) M. Levy, *J. Biol. Chem.*, **109**, 361 (1935).
(13) C. Tanford, S. A. Swanson and W. S. Shore, THIS JOURNAL, **77**, 6414 (1955).

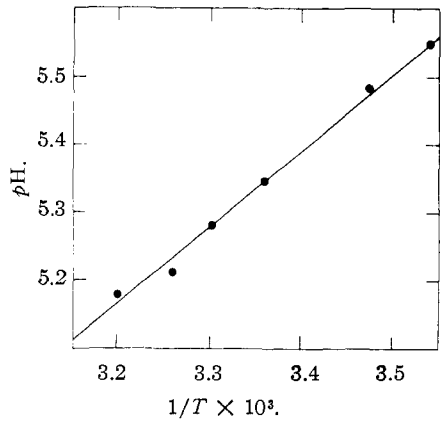


Fig. 3.—Variation of pH with temperature of a partially neutralized (α = 0.65) poly-L-histidine (n average 15) solution, at an ionic strength of 0.1 (NaCl).

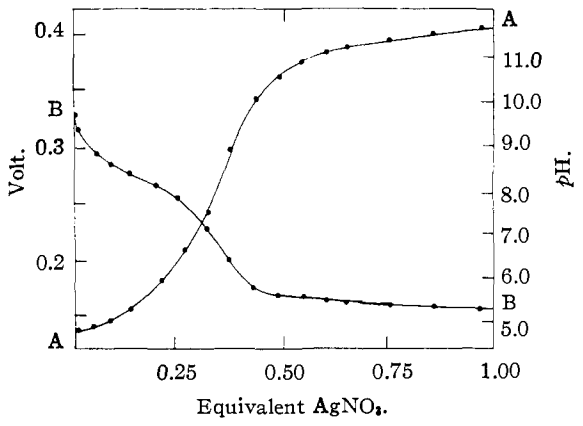


Fig. 4.—Electrometric titration of poly-L-histidine (n = 15) with silver nitrate at 25° and an ionic strength of 0.05 ($NaNO_3$). Curve A gives the silver electrode potential (in volts) against a calomel electrode. Curve B gives the corresponding pH values.

trode potential as a function of the amount of silver nitrate added. The inflection in the argentometric titration curve corresponds to the addition of approximately one-half equivalent of silver. The pH at this point coincides with that of polyhistidine at half neutralization.

Experimental

All melting points are uncorrected. L-Histidine was obtained from Nutritional Biochemicals Corporation.

1-Benzyl-N-carboxy-L-histidine Anhydride Hydrochloride (II).—1-Benzyl-N-carbobenzoxy-L-histidine[3] (3.8 g.) was suspended in anhydrous dioxane (5 ml.), and a solution of phosphorus pentachloride (2.2 g.) in dioxane (15 ml.) was added. The temperature of the reaction mixture was kept at 25° by cooling with tap water. After a few minutes a clear solution was obtained, and shortly thereafter crystals of the anhydride appeared. If spontaneous crystallization did not occur within 10 minutes, the solution was seeded with a few anhydride crystals obtained from a previous preparation. A prolonged delay in crystallization decreased the yield markedly. One hour after the onset of crystallization the crystals were collected, washed with dioxane and dried over phosphorus pentoxide and potassium hydroxide; yield 1.6 g. (52%), m.p. 187–189° dec.

Anal. Calcd. for $C_{14}H_{14}N_2O_3Cl$: C, 54.6; H, 4.6; N, 13.7; Cl, 11.5; mol. wt., 307.7. Found: C, 54.5; H, 4.8; N, 13.8; Cl, 11.4; mol. wt., 306, determined by anhydrous titration with 0.1 N sodium methoxide in dioxane using thymol blue as indicator[14] and assuming the presence of two titrable groups per molecule.

The anhydride hydrochloride II dissolves in dimethylformamide and in water (with decomposition) and is insoluble in dioxane, ethyl acetate, benzene and ether. A solution of II in dilute hydrochloric acid evolved carbon dioxide and yielded 1-benzyl-L-histidine quantitatively as determined by amino nitrogen analysis. A paper chromatogram of the acid solution developed with pyridine–water (65:35 by volume) showed only one ninhydrin positive spot with an R_f 0.83, identical with that of an authentic sample of 1-benzyl-L-histidine.[7] Crystals of 1-benzyl-L-histidine, m.p. 240°, were obtained from an acid solution brought to pH 8.0 to 8.5.

Poly-1-benzyl-L-histidine (III).—To a suspension of the anhydride II (20 g.) in anhydrous dioxane (200 ml.) was added triethylamine (10 ml.) in dioxane (50 ml.). The resulting precipitate of triethylammonium chloride was immediately filtered off. Polymerization proceeded at room temperature with an evolution of carbon dioxide and a precipitate of poly-1-benzyl-L-histidine appeared. After 24 hours at room temperature with magnetic stirring, the polymer was filtered, washed with dioxane and dried *in vacuo* over sulfuric acid. An average degree of polymerization, n = 50, was obtained by end-group analysis[5]; yield 11.8 g., $[\alpha]^{20}D$ −21.1° (c 2.0, in glacial acetic acid).

Anal. Calcd. for $(C_{13}H_{13}N_3O)_n$: C, 68.7; H, 5.8; N, 18.5; neut. equiv., 227.2. Found: C, 67.6; H, 5.9; N, 18.5; neut. equiv., 225, determined by anhydrous titration in dimethylformamide with 0.1 N perchloric acid in dioxane using thymol blue as indicator.

A second fraction of polymer (2.0 g.) of n = 20 was obtained from the mother liquor upon concentration and precipitation with water.

For diethylamine-initiated polymerizations the anhydride hydrochloride suspension in dioxane was first neutralized with an amount of triethylamine equivalent to the chloride present. After filtration the initiator was added and the polymerization carried out at room temperature. Monomer to initiator molar ratios of 20 to 100 yielded polybenzylhistidines with average degrees of polymerization n = 15 to 45.

Poly-1-benzyl-L-histidine, n = 50, obtained as above is soluble in glacial acetic acid, dimethylformamide and chloroform. It also dissolves in 0.1 N hydrochloric acid from which it is precipitated on neutralization. The precipitate formed can be redissolved in dilute hydrochloric acid, but is no longer soluble in dimethylformamide and chloroform. A polymer with similar solubility properties precipitates from a solution of poly-1-benzyl-L-histidine in dimethylformamide or chloroform upon heating.

Hydrolysis of III, n = 50, in 6 N hydrochloric acid at 120° for 24 hours yielded 1-benzyl-L-histidine quantitatively. The amount of benzylhistidine was estimated as described above.

A dilute solution of poly-1-benzyl-L-histidine in water containing one equivalent of hydrochloric acid gave precipitates with the following salts: NaCl, KI, KBr, KNO_3, $NaClO_4$, KI_3, KCN, $KMnO_4$, K_2SO_4, $Na_2S_2O_3$, $K_2S_2O_8$, K_2CrO_4, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$ and K_2HPO_4. No precipitate was obtained with KCNS. Solutions containing as little as 25 μg. of poly-1-benzyl-L-histidine per ml. gave visible precipitates in the presence of half molar solutions of KNO_3, $NaClO_4$ and $Na_2S_2O_3$. The precipitates of polybenzyl-L-histidine with nitrate, perchlorate, sulfate and persulfate were analyzed. A 0.1 N solution of poly-1-benzyl-L-histidine hydrochloride (5 ml.) was mixed with 3 ml. of an aqueous solution containing 1 mmole of KNO_3, $NaClO_4$, K_2SO_4 or $K_2S_2O_8$. The precipitates were centrifuged, washed four times with 4-ml. portions of water and dried at 70° *in vacuo* over phosphorus pentoxide. Titration of the polyvalent salts in dimethylformamide with 0.1 N sodium methoxide using thymol blue as indicator showed them to contain from 0.8 to 0.9 acidic equivalent per benzylhistidine residue. This was confirmed by chlorine analysis in the case of perchlorate and by sulfur analysis in the case of sulfate and persulfate.

Poly-L-histidine (IV).—To a suspension of poly-1-benzyl-L-histidine, n = 50 (1.0 g.), in anhydrous liquid ammonia

(14) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1554 (1953).

(40 ml.), contained in a reaction vessel fitted with a tube containing potassium hydroxide pellets, finely cut metallic sodium (0.1 to 0.2 g.) was added over a period of 15 minutes until a blue color persisted for at least 5 minutes. The excess of sodium was discharged with ammonium chloride, and the ammonia was allowed to evaporate spontaneously. The residue was dissolved in 1 *N* hydrochloric acid (15 ml.). This solution was extracted twice with 20-ml. portions of ether, and the ethereal extracts discarded. The aqueous layer was filtered and neutralized with 1 *N* sodium hydroxide. The precipitate which separated was centrifuged and washed with water until the washings were free of chloride. The poly-L-histidine obtained was dried *in vacuo* over sulfuric acid; yield 0.3 g., $[\alpha]^{22}D$ −20.0° (*c* 4.5, in glacial acetic acid).

The elementary analysis of the polymer, dried to constant weight *in vacuo* over phosphorus pentoxide at 100°, indicated the presence of approximately half a molecule of water per histidine residue.

Anal. Calcd. for $(C_6H_7ON_3)_n \cdot n/2H_2O$: C, 49.3; H, 5.5; N, 28.8. Found: C, 48.7; H, 5.9; N, 28.6.

Poly-L-histidine, $n = 50$, is soluble in dimethylformamide, glacial acetic acid, dichloroacetic acid and trifluoroacetic acid and is insoluble in ether, alcohol and acetone. It is soluble in dilute aqueous acids at *p*H values below 6 and in concentrated aqueous sodium hydroxide (above 3.5 *N*).

Hydrolysis of Poly-L-histidine.—Poly-L-histidine·$n/2H_2O$, $n = 50$, (0.571 g.) was refluxed in 6 *N* hydrochloric acid (6 ml.) for 18 hours and the hydrolysate diluted to 10 ml. with 6 *N* hydrochloric acid. A paper chromatogram of the hydrolysate, using pyridine–water (65:35 by volume) as the mobile phase gave only one spot with ninhydrin with an R_f 0.65, identical with that of an authentic sample of L-histidine. Analysis of the hydrolysate gave an amino nitrogen (Van Slyke) value of 9.1% (calcd. 9.6%), and a specific rotation of $[\alpha]^{22}D$ +14.0° (*c* 6.06, in 6 *N* hydrochloric acid), for the histidine present assuming a quantitative yield.[15]

Poly-L-histidine Hydrochloride.—Poly-L-histidine was dissolved in a slight excess of 2 *N* hydrochloric acid and the hydrochloride precipitated by acetone, washed with acetone and dried for several hours *in vacuo* at room temperature over solid potassium hydroxide and phosphorus pentoxide.

(15) M. S. Dunn, E. H. Frieden, M. P. Stoddard and H. V. Brown, *J. Biol. Chem.*, **144**, 487 (1942), give for L-histidine $[\alpha]^{25}D$ +13.3 (*c* 4.05, in 6.08 *N* hydrochloric acid).

Anal. Calcd. for $(C_6H_8N_3OCl)_n \cdot nH_2O$: C, 37.6; H, 5.3; N, 21.9; Cl, 18.5. Found: C, 37.5; H, 5.6; N, 22.3; Cl, 19.0.

Poly-L-histidine picrate was obtained by the addition of picric acid to an aqueous solution of poly-L-histidine hydrochloride. The picrate was washed with ether and dried *in vacuo* at 100° for four hours before analysis.

Anal. Calcd. for $(C_{12}H_{10}N_6O_8)$: C, 39.3; H, 2.8; N, 22.9; neut. equiv., 366; amino N (after acid hydrolysis), 3.8. Found: C, 39.2; H, 3.0; N, 21.9; neut. equiv., 364, determined by anhydrous titration in dimethylformamide with sodium methoxide using thymol blue as indicator; amino N, 3.9 (Van Slyke), after hydrolysis with 6 *N* hydrochloric acid for 48 hours at 120°.

Complexes of Poly-L-histidine with Metals.—The silver complex was prepared as follows: 5 *N* ammonium hydroxide (2 ml.) was added to a solution containing poly-L-histidine (100 mg.) and silver nitrate (500 mg.) in 0.2 *N* nitric acid (10 ml.). The precipitate formed was centrifuged and washed with dilute ammonium hydroxide until the washings were free of silver. The precipitate was then triturated with acetone and dried *in vacuo* over sulfuric acid. The precipitate contained approximately one atom of silver per histidine residue (found: Ag, 38.4).

The copper complex was prepared in an analogous manner from 100 mg. of poly-L-histidine and 500 mg. of copper sulfate in 10 ml. of 0.2 *N* sulfuric acid. The complex contained approximately one copper atom per histidine residue (found: Cu, 28.6).

Complexes of polyhistidine with zinc, magnesium and cobalt were prepared in the manner described for the silver complex from poly-L-histidine and the sulfates of zinc, magnesium and cobalt. They were found to contain 35.1% Zn, 11.3% Mg and 8.4% Co, respectively.

The mercury complex, containing 34.4% Hg, precipitated from an acid solution of poly-L-histidine in nitric acid upon the addition of mercuric nitrate.

Measurements of *p*H.—Measurements of *p*H were made on a model G Beckman *p*H meter. A standard phosphate buffer (*p*H 7.00) was used for calibration.

Argentometric Titrations.—The argentometric titrations were carried out with a model G Beckman *p*H meter using an uncoated silver wire as the indicator electrode.

Acknowledgment.—This investigation was supported by a research grant (H 2279) from the U.S.A. National Institutes of Health, Public Health Service.

REHOVOTH, ISRAEL

[CONTRIBUTION FROM THE CHEMICAL CORPS, FORT DETRICK, THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY,[1] AND THE GEORGE WILLIAMS HOOPER FOUNDATION, UNIVERSITY OF CALIFORNIA[1]]

Paralytic Shellfish Poison. VI. A Procedure for the Isolation and Purification of the Poison from Toxic Clam and Mussel Tissues

BY EDWARD J. SCHANTZ, JAMES D. MOLD, D. WARREN STANGER, JOHN SHAVEL, FRANK J. RIEL, JOHN P. BOWDEN, JOSEPH M. LYNCH, RUTH SAVAGE WYLER, BYRON RIEGEL AND HERMANN SOMMER

RECEIVED MAY 14, 1957

A procedure is described for the purification of the poisons from toxic clam and mussel tissues. It involves extraction of the toxic tissues with acidified aqueous ethanol, ion exchange fractionation on carboxylic acid resins (Amberlite IRC-50 and XE-64) and chromatography on acid-washed alumina. The purified poison from either clams or mussels has a toxicity of 5500 ± 500 average lethal mouse doses per mg. and a specific rotation of +130 ± 5°.

The procedures described previously[2] for the purification of the poison from toxic mussels (*Mytilus californianus*) or from the plankton *Gonyaulax catenella* yielded preparations with toxicities up to about 1600 mouse units (MU)[3] per mg. An improved procedure described in this paper resulted in the purification of mussel poison to a toxicity of

(1) The work performed in these laboratories was carried out under contract with the Chemical Corps.

(2) (a) H. Sommer, R. P. Monnier, B. Riegel, D. W. Stanger, J. D. Mold, D. M. Wikholm and E. S. Kiralis, THIS JOURNAL, **70**, 1015 (1948); (b) H. Sommer, B. Riegel, D. W. Stanger, J. D. Mold, D. M. Wikholm and M. B. McCaughey, *ibid.*, **70**, 1019 (1948); (c) B. Riegel, D. W. Stanger, D. M. Wikholm, J. D. Mold and H. Sommer, *J. Biol. Chem.*, **177**, 7 (1949).

(3) The toxicity values given in this paper were determined by the intraperitoneal injection of 1.0 ml. of aqueous solutions of the poison into white mice. The mouse unit is the average lethal dose of poison that will kill a 20 g. mouse in 15 minutes (see Experimental section).