(5) K. H. Beyer, *Ann. N. Y. Acad. Sci.*, **71**, 363 (1958).
(6) B. Johnson, *Clin. Res.*, **17**, 433 (1969).
(7) J. F. Mustard, B. Hegardt, H. C. Rowsell, and R. L. MacMillan, *J. Lab. Clin. Med.*, **64**, 548 (1964).
(8) R. D. MacKenzie, T. R. Blohm, and E. M. Auxier, *Amer. J. Clin. Pathol.*, **55**, 551 (1971).
(9) S. Goldstein and M. Schnall, *Arch. Int. Pharmacodyn. Ther.*, **144**, 269 (1963).
(10) J. H. Birnie, B. M. Sutton, M. Zuccarello, and J. A. Rush, *Med. Pharmacol. Exp.*, **17**, 51 (1967).
(11) H. Schnell and J. Nentwig, "Houben-Weyl, Methoden der Organischen Chemie," Vol. 11, Part 2, E. Müller, Ed., Georg Thieme Verlag, Stuttgart, 1958, pp 577-578.
(12) R. Kwok and P. Pranc, *J. Org. Chem.*, **32**, 738 (1967).
(13) R. E. Benson and T. L. Cairns, *J. Amer. Chem. Soc.*, **70**, 2115 (1948).
(14) H. Bredereck and K. Bredereck, *Chem. Ber.*, **94**, 2278 (1961).
(15) H. Bredereck, F. Effenberger, and G. Simchen, *ibid.*, **97**, 1403 (1964).
(16) L. P. Cawley, F. E. Spear, and R. Kendall, *Amer. J. Clin. Pathol.*, **32**, 195 (1959).
(17) O. Schales and S. S. Schales, *J. Biol. Chem.*, **140**, 879 (1941).

Synthesis of α,β-Poly[(2-hydroxyethyl)-DL-aspartamide], a New Plasma Expander

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Poly(amino acids) are of potential interest for use as plasma expanders, owing to their protein-like nature and the possibility of preparation by synthesis. High molecular weight poly-DL-succinimide was synthesized by thermal polyermization of aspartic acid in the presence of phosphoric acid, under reduced pressure, with some modifications of earlier methods. Reaction of this polymer with ethanolamine in controlled conditions afforded α,β-poly[(2-hydroxyethyl)-DL-aspartamide], with a molecular weight between 10,000 and 90,000. The poly(amino acid) is water-soluble, nontoxic, and nonantigenic in animals; moreover, the effect on the clotting time is less than in the case of dextran and the clearance rate lower than that of gelatin. For these reasons its use as a plasma expander is proposed. The difficulties of scaling up the method for the practical preparation of a plasma expander are considerably less than those involved in the preparation of an analogous compound [poly[N^5-(2-hydroxyethyl)-L-glutamine]] previously proposed by us.

The use of poly(amino acids) as plasma substitutes has been proposed by various authors in the past.[1-6] Poly(amino acids) would seem to have definite advantages over the other polymers generally used for this purpose; in fact, these compounds are similar to proteins and therefore one could assume that they will be cleaved in the body to amino acids or small peptides, which would be easily eliminated and nontoxic, and also able to contribute to the patient's nutrition. In order to be used as a plasma substitute, a poly(amino acid) must satisfy some fundamental requirements, *e.g.*, lack of toxicity and immunogenicity. Moreover, the molecular weight of a product to be used as a plasma expander should be low enough to ensure a maximum of oncotic pressure per weight unit and at the same time be sufficiently high to be retained in the blood to exert a steady pharmacological effect. However, other factors such as the shape of the molecules and the chemical nature of the compound can affect both the magnitude and the duration of the activity.

The first poly(amino acid) proposed as a possible plasma expander was poly(glutamic acid).[3-6] However, the results were not encouraging, because this compound proved to be either inefficient[3,4] or toxic.[5,6] One can speculate that this inconvenience was related to the high net charge exhibited by this compound at physiological pH,[5,6] since the copolymers of glutamic acid and lysine, for instance, were found to have a lower toxicity.[7] Consequently, we decided to prepare poly(amino acids) with no charge for use as plasma expanders. This can be achieved easily by blocking the side carboxyl groups in an amide linkage with an amino alcohol. The hydroxyl would render the polymer soluble in water, at the same time eliminating the electric interaction with cells and other components of the organism.

We have previously shown[8] that poly[N^5-(2-hydroxyethyl)-L-glutamine (PHEG)[†] has proved efficient, nontoxic, and nonimmunogenic when tested in animals. However, the large-scale preparation of this product raises complex technical and economic problems. This is a serious disadvantage for a plasma expander, one of the fundamental requirements for this kind of product being easy, low-cost production.[9] A plasma expander with better characteristics than those of existing products would hardly be of importance if it could not be easily produced at a reasonable cost and in large quantities. In this respect, the synthesis of analogous derivatives of poly(aspartic acid) is of particular interest. In fact, these compounds are easily obtained by polymerization of aspartic acid simply by heating[10-13] to yield a polysuccinimide [anhydropoly(aspartic acid)] reactive with amines.[14] Thus, compounds similar to poly(hydroxyalkylglutamines) are obtained after reaction with amino alcohols.

The methods described in the literature for the polymerization of aspartic acid provide polymers of low molecular weight, however, which are unsuitable for the synthesis of a plasma substitute.

By polymerizing aspartic acid in the presence of phosphoric acid in thin layer under reduced pressure, we obtained a polysuccinimide which gave α,β-poly[(2-hydroxyethyl)-DL-aspartamide] with a molecular weight of about 50,000 when reacted with ethanolamine, as shown in Scheme I.

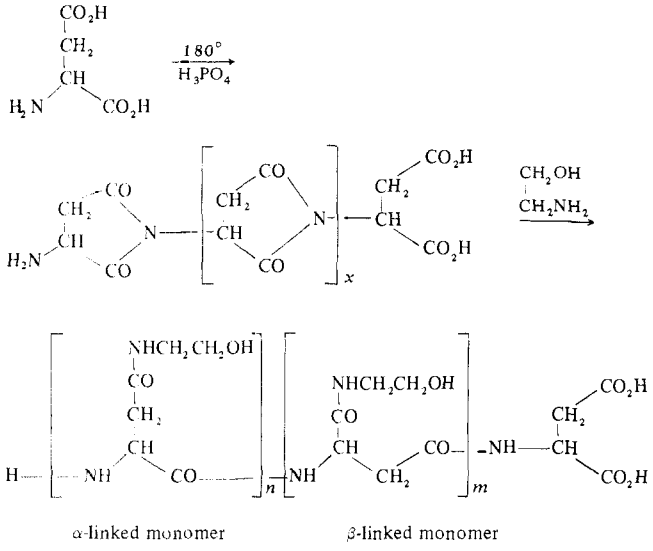
While the synthesis of PHEG involves at least four steps (synthesis of γ-methylglutamate, *N*-carboxy anhydride formation, polymerization, and reaction with ethanolamine) the preparation of PHEA requires only two, simpler reactions. In addition, the management of large amounts of phosgene, a reagent widely used for the synthesis of *N*-carboxy anhydride, raises complex problems as regards safety in a large-scale plant. On the contrary, experience so far gained in a pilot plant has shown that the preparation of PHEA can be scaled up economically.

Results and Discussion

Polymerization of Aspartic Acid. Thermal polymerization of DL-aspartic acid in the presence of H_3PO_4 and in

[†] Abbreviations: PHEA, α,β-poly[(2-hydroxyethyl)-DL-aspartamide]; PHEG, poly[N^5-(2-hydroxyethyl)-L-glutamine]; DCC, *N,N'*-dicyclohexylcarbodiimide; DMF, *N,N*-dimethylformamide.

Scheme I



thin layer produces poly-DL-succinimide with a high degree of polymerization. However, much lower degrees of polymerization are obtained by polymerization without H_3PO_4.

Table I shows the reduced viscosities (at 25°, *c* 0.5% in DMF) measured on the polymer prepared by us according to methods reported in literature.

We have also reported, as an indication, the degree of polymerization, *n*, calculated by applying to the polymer the formula, $n = 3.52 \cdot \eta_{red}^{1.56}$, calculated from the equation, $[\eta] = 1.48 \cdot 10^{-2} \cdot M^{0.64}$ of poly(β-benzyl-DL-aspartate).[15]

The method according to Vegotski, *et al.*,[11] consists of simply heating aspartic acid for 2-4 hr at 200° in an open tube; the molecular weight reported by these authors, as measured by the dinitrofluorobenzene method, is about 11,000 (DP ≅ 110). Later Kovacs, *et al.*,[12] prepared the polymer by heating aspartic acid at 200° for 120 hr in a high vacuum or in boiling tetraline for 100 hr with azeotropic removal of water.

We did not find any substantial advantage in using either one or the other method for the polymerization of aspartic acid. Fox and Harada[13] demonstrated that the addition of H_3PO_4 to the aspartic acid leads to improved yields and allows the polymerization to be carried out at a lower temperature. We observed that the use of H_3PO_4 also leads to a distinct improvement in the degree of polymerization.

The best results are obtained by effecting the polymerization in thin layer, in an oven at a temperature of about 180°; it is possible to obtain a polymer of very high reduced viscosity by this method; by carrying out the polymerization at reduced pressure there is a continual renewal of the free surface of the polymer and a polysuccinimide can be obtained of a viscosity up to 50 ml/g.

Fox and Harada[13] reported that the best yields are obtained with 50 ml of 85% phosphoric acid per mole of aspartic acid (molar ratio about 3:4). We found that smaller amounts of phosphoric acid gave results that were notably better. On the other hand, the same authors found that in the copolymerization of aspartic acid with glutamic acid (2 mol of aspartic and 1 mol of glutamic acid) the best yields were obtained with 2 mol of phosphoric acid to 3 mol of total amino acids. Table II reports the results obtained by us, using different amounts of H_3PO_4.

The degree of polymerization can be notably increased by using dicyclohexylcarbodiimide. Figure 1 reports the reduced viscosities of the polymer after the addition of increasing amounts of dicyclohexylcarbodiimide. DCC in amounts of about 20 mg/g of polymer causes the greatest increase in viscosity. Larger amounts do not cause a further increase; in fact, there is a certain tendency to a decrease in the reduced viscosity. In order to ascertain the time required for the reaction with DCC, tests were carried out using reaction times of 4, 8, 24, and 72 hr; the results were always approximately the same.

Table I. Degree of Polymerization, *n*, of Poly-DL-succinimide According to Polymerization Conditions, as Deduced from Viscosity Measurements

Polymerization conditions	Ref	η_{red}	*n*
4 hr at 200°, atmospheric pressure	11, 13	10	130
100 hr at 200°, *in vacuo*	12	10	130
100 hr in boiling tetraline with azeotropic removal of water	12	10	130
4 hr at 170° in presence of H_3PO_4, at atmospheric pressure	13	15	240
4 hr at 180°, in presence of H_3PO_4, at atmospheric pressure, in thin layer		20–35	380–900
2.5 hr at 180°, in presence of H_3PO_4, *in vacuo*, in thin layer		40–50	1100–1600
In rotary evaporator, in presence of H_3PO_4, 2.5 hr at 180° *in vacuo*		40	1100

Table II. Reduced Viscosity of the Polysuccinimide According to the Amount of Phosphoric Acid Used for Polymerization

Phosphoric acid:aspartic acid molar ratio	η_{red} (0.5%, DMF), ml/g
0.0	7–10
0.4	22
0.6	45
0.8	42
2.0	28

Reaction with Ethanolamine. The polymer is very reactive with organic and inorganic bases.[10–14] We obtained α,β-(poly-DL-aspartic acid) by reaction with dilute NaOH; we prepared α,β-poly[(2-hydroxyethyl)-DL-aspartamide], potentially useful as a plasma expander, by reaction with ethanolamine.

The reaction of the polysuccinimide with ethanolamine is strongly exothermic and takes place simply by mixing the reagents, even in the absence of solvents. It is advisable, however, to use the mildest possible reaction conditions to

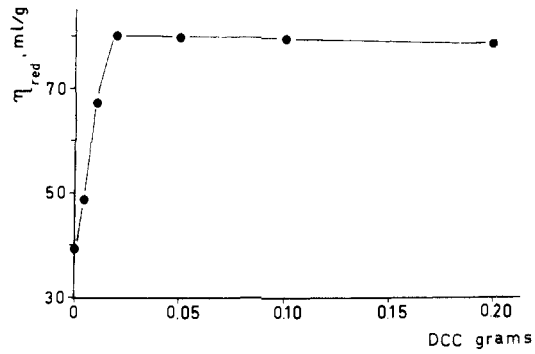


Figure 1. Increase in reduced viscosity of the polysuccinimide following the reaction of 1 g of polymer with varying amounts of DCC. Viscosities were determined on samples of the polymer isolated from the reaction mixture.

Table III. Molecular Weight of Poly(hydroxyethylaspartamide) According to the Preparation Method

Reduced viscosity of original polysuccinimide, ml/g	Amt of ethanolamine used for the reaction, ml/g of polysuccinimide	Reaction time, hr	Temp, °C	M
40	50	1	50	10,000-20,000
40	1.5	2	30	40,000-50,000
80	50	1	30	60,000-80,000
80	1.5	2	30	80,000-90,000

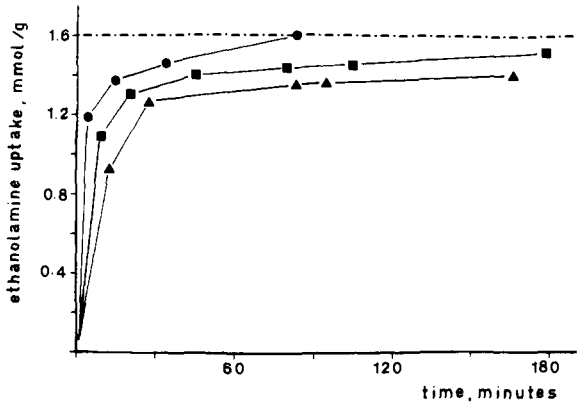


Figure 2. Rate of ethanolamine uptake by polysuccinimide in DMF. The consumption of ethanolamine is calculated as the difference between the total amount initially added to the solution and the amount actually present in solution at the time t. Concentrations are expressed as millimoles of solute per gram of total solution. The initial concentration of polysuccinimide is 1.60 mmol of monomer per gram of solution. The experiments were carried out with initial amounts of ethanolamine in excess with respect to the stoichiometric quantity. The following ethanolamine:succinimide monomer molar ratios were used: ●, 1.70; ■, 1.37; ▲, 1.14.

obtain high molecular weights; in fact, the excess ethanolamine may cause partial degradation of the polypeptide chain, in addition to opening the succinimide rings. The temperature and reaction time had a clear effect on the characteristics of the product obtained; too long a contact between the reagents or too high a reaction temperature, over 30-40°, always resulted in a product of low molecular weight (see Table III).

It was interesting, therefore, to ascertain the minimum amount of ethanolamine necessary to have a complete reaction in a reasonably short period. For this purpose, solutions of polysuccinimide were prepared (η_{red} = 40 ml/g) in DMF (c 0.2 g/ml) and allowed to react with different amounts of ethanolamine at 25°.

Samples were isolated from the reaction mixtures after various lengths of time; the samples were weighed accurately, neutralized with dilute HCl, and analyzed colorimetrically with ninhydrin[16] to determine the amount of free ethanolamine still present (Figure 2).

By using 1.7 mol of ethanolamine per mole of polysuccinimide monomer a complete reaction was apparently obtained after about 80 min. In actual fact, by carrying out analogous experiments with larger amounts of polysuccinimide, and isolating the reaction product at various times, it could be seen that the reaction was not complete even after 2 hr in the conditions described above.

In fact, by potentiometric titration it could be seen that the samples isolated all showed a slow uptake of the base at alkaline pH with a subsequent decrease in pH. This is presumably related to the fact that the polymer isolated in these conditions still contains succinimide monomers which react with the base during the titration.

However, when 2.4 mol of ethanolamine per mole of polysuccinimide monomer were used, the reaction was complete in less than 2 hr; the chemical characteristics of this preparation were in good agreement with the theoretical values.

Potentiometric titration with acids demonstrated the presence of about 0.08 mol of free carboxyl per gram of polymer. For a molecular weight of 50,000, this is equivalent to the presence of four free carboxyls for each polymeric chain. Since no steps were observed in the titration curves in the pH range from 7 to 12, it can be inferred that no side groups (such as succinimide or oxazoline rings)[17,18] were present apart from those expected.

Molecular Weight. The molecular weight of poly(hydroxyethylaspartamide) determined by ultracentrifugation varies from 10,000 to 90,000 according to the reduced viscosity of the original polysuccinimide and the conditions adopted for the reaction with ethanolamine (see Table III). The relationship between viscosity and molecular weight fits the equation, $[\eta] = 5.3 \cdot 10^{-4} M$; the exponent value of the Mark-Howink equation is close to 1, corresponding to a semirigid structure of the polymer.[19] A more detailed report of the hydrodynamic properties of PHEA will be published elsewhere. Under the conditions adopted, it is possible to obtain a polyamide with a molecular weight of about 40,000 or about 80,000 according to whether or not the original polysuccinimide was treated with DCC.

Pharmacology. PHEA differs widely from PHEG in terms of general structure, since in the former polymer, peptide linkages of the β type are present, in addition of those of the α type, the only one existing in the polyglutamic derivative, and owing to the presence of amino acid residues in the D configuration. This fact is reflected by the resistance of PHEA to proteolysis with papain,‡ in comparison with the hydrolysis undergone by PHEG.[8] Resistance to proteolysis is in turn of importance in determining the rate of excretion of these polymers through a preliminary breakdown of the whole molecule into smaller peptides or amino acids. Excretion should be slow enough to allow the compound to exert a prolonged expansion of the circulating volume without being noticeably accumulated. This is the case as regards PHEA, as we have found that 40% of the amount of PHEA originally infused was retained in rabbits after 8 hr. This value fell to 20% after 24 hr, while about 50% was recovered in the urine collected over the same period (Figure 3).

These findings are of importance, bearing in mind that one of the most serious disadvantages of gelatin and derivatives,[20] a class of plasma expanders widely used in Europe, is the rapid clearance from the body, resulting in turn in a short duration of the effect.

Like PHEG, PHEA lacks toxicity and antigenicity. In fact, it was not possible to determine an LD_{50} in mice, owing to the extreme tollerability in the rat over a period of 40 days. Daily iv injections of the compound at doses ten times greater than those likely to be used in man did not induce any significant changes in total weight gain or organ weight. The blood sugar, urea, sodium, and potassium levels were unaffected, and neither were those of GOT, GPT, and phosphatases. Moreover, no pathological changes were detected in histological examiniation of various organs. The electrophoretic pattern of the serum proteins and the blood cell

‡P. Neri, *et al.*, unpublished results.

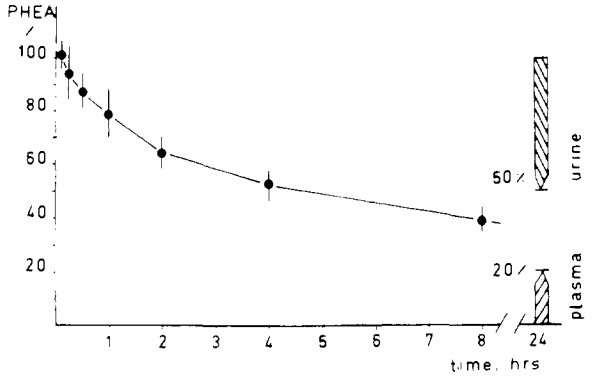


Figure 3. Rate of disappearance of PHEA from rabbit plasma. Rabbits were infused with 7.0 ml/kg of PHEA, 4.5% (average molecular weight 54,000), after bleeding of 5.5 ml/kg. The percentage of the initial amount infused, found in the plasma at various times, is reported in the ordinate. PHEA was determined by measuring the amount of ethanolamine freed by acid hydrolysis from the plasma fraction not precipitated by trichloroacetic acid.

Table IV. Effect on Blood Clotting Time in the Cat

Experimental groups	Changes in clotting time,[a] % Immediately after bleeding	3 hr after bleeding
Saline	−17	+2
Dextran	−5	+57
Gelatin (cross-linked)	−6	−14
PHEA	−15	+10

[a] Differences from standard values measured before bleeding.

Table V. Effect on Erythrocyte Sedimentation Rate in the Cat

Experimental groups	Dose, ml/kg	Values in mm ± S.E.[a]
Standard values		4.4 ± 0.6
Saline	14	3.8 ± 0.7
PHEA, 3%	14	47.4 ± 7.0[b]
Dextran, 6%	14	124.3 ± 4.2

[a] Values measured 3 hr after bleeding. [b] Highly significant difference ($0.00 < F < 0.005$).

count appeared normal at the end of treatment, indicating that poly(hydroxyethylaspartamide) has no adverse effect on the biosynthetic mechanism of the serum proteins or of the blood cells under the experimental conditions adopted. As in the case of poly(hydroxyethylglutamine), we observed no adverse effect on the cardiac output in the cat.

In order to establish the possible immunogenic activity of the compound, PHEA was injected in rabbits and guinea pigs according to a wide number of immunization patterns which differed in only one parameter, such as the injection route (iv or sc), the amount of compound injected (from a minimum of 1 μg to 216 mg), the number of injections (from 1 to 6), or the period of time over which bleeding took place. Freund's complete adjuvant was added in some patterns in order to obtain the most favorable conditions for the formation of antibodies. The sera from the bled animals were tested for the presence of precipitating antibodies by microdiffusion in agar plates.

The presence of sensitizing antibodies was assayed by the test according to Ovary[21] in guinea pig sera, and changes in the arterial pressure after injection of a booster dose were measured in the rabbit.

No evidence of an immune response was found in any of the immunization patterns adopted. We can conclude that PHEA possesses no evident antigenic activity. This result is important, in view of the fact that a certain antigenicity

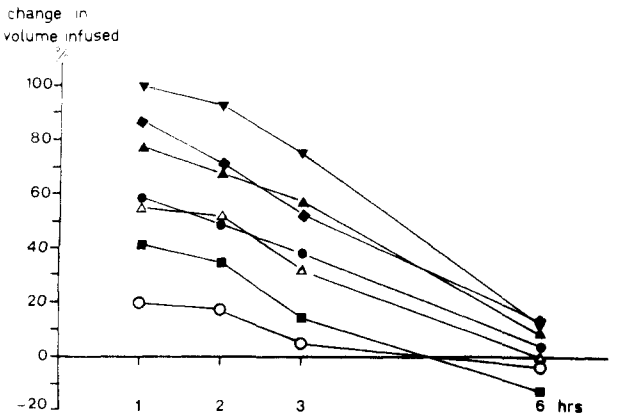


Figure 4. Changes in plasma volume following the infusion of PHEA, saline, and various expanders in the cat: ○, saline; ●, plasma; ◆, dextran, 6.0%, average molecular weight 70,000; ■, cross-linked gelatin, 3.5%, average molecular weight 35,000; △, PHEA, average molecular weight 54,000, 3%; ▲, PHEA, average molecular weight 54,000, 4.5%; ▼, PHEA, average molecular weight 54,000, 6.0%.

has been claimed for dextran,[22] at present the most widely used plasma expander.

PHEA has another advantage over dextran, *i.e.*, that of not noticeably increasing the coagulation time, as shown in Table IV. On the other hand, PHEA has a noticeable effect on the erythrocyte sedimentation rate in the cat, as shown in Table V. However, this effect is much less marked than that produced by dextran 70, which greatly increases the esr in the same experimental conditions.

Finally, PHEA has proved to be as active as other plasma expanders in maintaining an infused volume of expander after bleeding, as shown in Figure 4. The effect was determined in the cat by the method of radiodilution of serum proteins labeled with ^{125}I. A 4.5% solution of PHEA, molecular weight 54,000, has the same effect as that of 6% dextran and greater than that produced by a 3.5% solution of cross-linked gelatin.

Experimental Section

The reagents were all commercial products of reagent grade, used without further purification. Viscosities were recorded at 25° with an Ostwald viscosimeter, with a flow time of 100–200 sec for water. The viscosities of samples of poly-DL-succinimide were measured in DMF at a concentration of 0.5%; the viscosities of samples of poly(hydroxyethylaspartamide) were measured in aqueous solution at a concentration of 0.5%. The results were expressed as reduced viscosities.

$$\eta_{red} = (\eta - \eta_0)/\eta_0 C \text{ (ml/g)}$$

The intrinsic viscosities of some samples of poly(hydroxyethylaspartamide) were also tested by measuring the reduced viscosity at different concentrations and extrapolating the value at zero concentration.

The titration curves were determined in KCl (0.2 *N*) with a glass electrode, pH meter Metrohm Herisau, Precision Potentiometer E 353 B (sensitivity ±0.01).

The elementary analyses were carried out by the Mikroanalytisches Laboratorium, Elbach über Engelskirchen, West Germany).

The molecular weight measurements of the poly(hydroxyethylaspartamide) were carried out by Dr. M. Ottesen of the Carlsberg Laboratorium. The sedimentation equilibrium method was used with samples of polymer at different intrinsic viscosities. A Spynco Model E analytical ultracentrifuge was used.

Thermal Polymerization of Aspartic Acid. Method A. Polymerization in Rotary Evaporator. Finely ground aspartic acid (50 g) was mixed with 25 g of 85% phosphoric acid in a 2-l. flask. The flask was placed in a rotary evaporator and heated for 2.5 hr under reduced pressure in an oil bath at 180°. The vitreous mass obtained was dissolved in 200 ml of *N,N*-dimethylformamide and the solution was poured into a beaker containing 1 l. of water. A flaky precipitate

formed which was filtered, rinsed with water until neutrality, and dried in an oven at 110° for 24 hr, yield 35 g (96%). *Anal.* ($C_4H_3NO_2$) C, H, N, O. The polymer must be quantitatively dried before analysis to obtain good analytical values.

Method B. Polymerization in Thin Layer. Aspartic acid (100 g) was mixed with 50 g of phosphoric acid and the resulting pasty mass was spread on a Teflon-covered tray of about 1000 cm^2. The tray was placed in a vacuum oven and heated to 180° for 2.5 hr under reduced pressure. The polymer was isolated as previously described; the yield and analytical characteristics were the same.

Condensation with Dicyclohexylcarbodiimide. Poly-DL-succinimide (1 g) was dissolved in 5 ml of DMF; DCC (50 mg) was added to the solution and the mixture stirred for 24 hr at room temperature. The solution was then filtered in order to eliminate the dicyclohexylurea and the poly-DL-succinimide precipitated by the addition of water. The precipitate was rinsed with water and ethanol and dried at 110° for 24 hr, yield quantitative. The product has the same analytical characteristics as the original poly-DL-succinimide. Similar experiments were carried out using different amounts of DCC to establish the amount to be used to obtain the highest molecular weight (see Results and Discussion).

Reaction with Ethanolamine. Poly-DL-succinimide (30 g) was dissolved in 150 ml of DMF. Ethanolamine (45 ml) was then added drop by drop and the solution cooled in an ice bath to keep the temperature at 25–30°. The mixture was stirred for 2 hr and then neutralized with glacial acetic acid (about 30 ml), diluted with water, dialyzed, and lyophylized, yield 42 g (86%). *Anal.* ($C_6H_{10}N_2O_3$) C, H, N, O. The product obtained by this method had an average intrinsic viscosity of 25 ml/g; the molecular weight, determined in the ultracentrifuge, was about 50,000.

The reaction was also carried out using polysuccinimide treated with DCC. The product obtained had the same analytical characteristics as the previous one. The intrinsic viscosity was about 40 ml/g and the molecular weight about 80,000.

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References

(1) M. Sela and E. Katchalski, *Advan. Protein Chem.*, 14, 391 (1959).
(2) M. A. Stahmann, "Proceedings of the International Symposium on Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p 329.
(3) M. Bovarnick, S. Fieber, M. R. Bovarnick, and J. Kozlowski, *Proc. Soc. Exp. Biol. Med.*, **83**, 253 (1953).
(4) B. J. Kessler, C. G. Di Grado, C. Benante, M. Bovarnick, R. H. Sieber, and A. J. Zambito, *ibid.*, **88**, 651 (1955).
(5) A. D. Kenny, *ibid.*, **100**, 778 (1959).
(6) W. Y. Loebl, T. D. Ullmann, A. Yaron, M. Sela, A. Berger, and E. Katchalski, *ibid.*, **108**, 661 (1961).
(7) E. R. Blout, S. Farber, G. D. Fasman, E. Klein, and M. Narrod, ref 2, p 379.
(8) A. Gerola, G. Antoni, F. Benvenuti, F. Cocola, and P. Neri, "Shock: Biochemical, Pharmacological and Clinical Aspects," Plenum Press, New York, N. Y., 1970, p 329.
(9) U. F. Gruber, "Blood Replacement," Springer-Verlag, Berlin-Heidelberg-New York, 1969, p 51.
(10) J. Kovacs, I. Konyves, and A. Pusztai, *Experientia*, 9, 459 (1953).
(11) A. Vegotsky, K. Harada, and S. W. Fox, *J. Amer. Chem. Soc.*, **80**, 3361 (1958).
(12) J. Kovacs H. N. Kovacs, I. Konyves, J. Csaszar, T. Vajda, and H. Mix, *J. Org. Chem.*, **26**, 1084 (1961).
(13) S. W. Fox and K. Harada, "A Laboratory Manual of Analytical Methods of Protein Chemistry, Including Polypeptides," P. Alexander and H. P. Lundgren Ed., Pergamon Press, Elmsford, N. Y., 1966, p 127.
(14) H. N. Kovacs, J. Kovacs, M. A. Pisano, and B. A. Shidlovsky, *J. Med. Chem.*, **10**, 904 (1967).
(15) G. Spach, Thesis, Strasbourg, 1960; "Polyamino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, p 147.
(16) H. Fraenkel-Conrat, *Methods Enzymol.*, 4, 252 (1957).
(17) N. Lupu, A. Yaron, M. Sela, and A. Berger, *Bull. Res. Counc. Isr., Sect. A., Chem.*, **10**, 47 (1961).
(18) N. Lupu-Lotan, A. Yaron, A. Berger, and M. Sela, *Biopolymers*, **3**, 625 (1965).
(19) J. T. Yang *Advan. Protein Chem.*, **16**, 323 (1961).
(20) Reference 9, p 132.
(21) Z. Ovary, "Immunological Methods," J. F. Ackroyd, Ed., Blackwell, Oxford, p 259.
(22) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," Macmillan, New York, N. Y., 1970, p 786.

Long-Acting Delivery Systems for Narcotic Antagonists. 1†,‡

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The relationship between the release rate of cyclazocine from composites with poly(lactic acid) and (a) the molecular weight of the polymer and (b) the form of the composite, as a film sealed in an envelope or as discrete small particles, has been investigated *in vivo* and *in vitro*. The release rate is not very sensitive to variations in the molecular weight of the polymer within the values investigated. As may be expected, the lower molecular weight polymer is absorbed faster than the higher molecular weight polymer. The use of the composite as a film sealed in an envelope of pure polymer permits control of the release rate. Desirable delivery rates have been obtained by injecting suspensions of small particles of the composite thereby eliminating the necessity of surgery. In experiments with films, the release rate of cyclazocine *in vivo* is faster than *in vitro*, whereas in experiments with small particles a reverse effect is observed.

In the past few years we have been interested in developing a method of delivering narcotic antagonists to a patient at a constant rate over a prolonged period, perhaps as long as several months. The migration of drugs through waxes, ointments, or polymers has been the object of various investigations.[1-3]

Composites of radioactive cyclazocine (2-cyclopropylmethyl-2′-hydroxy-5,9-dimethyl-6,7-benzomorphan) with polymeric materials in film or very small particle form were selected for this study. The delivery rate of the antagonist was determined by surgically implanting or hypodermically injecting these composites into rats and measuring the radioactivity of urinary excretion. A control experiment

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