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ADVANCES IN THE SYNTHESIS OF POLYPEPTIDES

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I. INTRODUCTION

Among polyamides there is a large group of polymers which differ from other polyamides in their properties and methods of preparation. This group, called polypeptides or poly-α-amino-acids, is very close in its composition and structure to one of the most important classes of polymeric substances — proteins. In contrast to proteins in which there is a strictly defined alternation of amino-acids, synthetic polypeptides usually contain only one amino-acid or several amino-acids with a random distribution along the chain of the macromolecule.

By polycondensation of various low-molecular-weight derivatives of peptides, it proved possible to prepare block-polypeptides with a random distribution of the blocks but with a known alternation of the amino-acids within each block, and also polypeptides with a defined alternation of amino-acids, whose number however was small.

$$[-HNCHR_1COHNCHR_2CO-]_x[-HNCHR_3COHNCHR_4CO-]_y \text{ and}$$
$$[-HNCHR_1COHNCHR_2COHNCHR_3CO-]_n.$$

Starting with the work of Fischer [1], Bergmann [2], and Leuchs [3], studies of the synthesis and properties of peptides and polypeptides have proceeded on a wide front. The results of the investigations published up to 1957 were described in detail previously [4,5]. The further increase in the number of papers published, including reviews, on the individual problems of the chemistry of polypeptides [6-13] indicates the growing importance of synthetic polypeptides.

One of the main aims of protein chemistry in the last decade has been the elucidation of the correspondence between biological functions and the chemical structure of proteins [14]. Synthesis of polypeptides as model compounds is one of the possible ways of determining the effect of various amino-acids, their sequence, and steric configuration on biological properties.

Synthetic polypeptides are also of considerable interest for polymer chemistry, since the methods of their preparation permit nowadays the synthesis of polymers with various amino-acid compositions and different structures, and with molecular weights ranging from several hundred to a million and above, and also they provide a means of modelling natural peptides, and modifying their composition, structure, and properties. This makes it possible to obtain polymers whose properties vary within very wide limits, and thereby solve important problems in the chemistry of macromolecular compounds.

In view of the abundance of data available, we are obliged to restrict ourselves to a description of the principal results of studies of the synthesis of polypeptides, published between 1957 and 1963, including certain important sections such as the chemical reactions and conformational analysis of polypeptides and also the methods of investigating their structures. Furthermore, the review does not deal with the important field of stepwise syntheses of peptides and their application, which is of independent interest. The review considers in detail the synthetic methods which have been most extensively developed in the last six years.

II. SYNTHESIS OF POLYPEPTIDES FROM α-AMINO--ACIDS, PEPTIDES, AND 2,5-DIOXOPIPERAZINE DERIVATIVES

1. Thermal Polycondensation of α-Amino-Acids and Peptides

It has been found that the usual method of polycondensation of amino-acids containing an amino-group at some distance from the carboxy-group [$H_2N(CH_2)_nCOOH$, $n \geq 5$] cannot be successfully used with α-amino-acids and peptides. At elevated temperatures the amino-acids and peptides undergo various side reactions (decarboxylation, deamination, cyclodehydration, etc.) resulting in the formation of low--molecular-weight products. Furthermore, the closeness of the amino- and carboxy-groups in α-amino-acids favours the formation at high temperatures of 2,5-dioxopiperazines, dimeric cyclic derivatives with high stability [15]. 2,5-Dioxopiperazines are also formed as a result of the degradation of polypeptides [16-18] on prolonged exposure to high temperatures. All these facts make it extremely difficult to obtain linear polypeptides directly from α-amino-acids. However, attempts to simplify to the greatest possible extent the synthesis of polypeptides are continued and to this day attract the attention of many investigators.

Among the various amino-acids studied, glycine undergoes polycondensation most readily. Meggy [19] suggested that, since glycine can be dehydrated at 140–180° in glycerol or ethylene glycol with the formation of 2,5-dioxopiperazine and the latter is thermodynamically less stable over a wide temperature range (60°–180°) than polyglycine, direct condensation of glycine to polyglycine is quite possible. Indeed, when glycine is heated with a small amount of hydrochloric acid in sealed tubes at temperatures in excess of 140°, it is converted into polyglycine, the yield of the polymer varying with the glycine : hydrochloric acid ratio.

Polyglycine is not obtained below 130° and when tubes which had been heated to 140° are kept a further 24 h at 130° the yield of the polymer falls appreciably. It appears that this reaction requires a temperature above 140° and the presence of a solid phase. Apart from hydrochloric acid, the reaction is catalysed by phosphoric acid, ammonium chloride, and sodium hydroxide. It can also be carried out in the presence of water alone, but hydrochloric acid ensures the highest yield and the best quality of the polymer.

Meggy believes that the polymerisation of glycine may be regarded as a heterogeneous stepwise reaction between glycine in solution and the solid polyglycine:

$$\text{glycine} + \text{X-mer} = (\text{X} + \text{I})\text{-mer} + H_2O. \qquad (1)$$

It would appear that this process could result in prolonged growth of the polymeric chain and hence in a prolonged increase of the average degree of polymerisation n. However, it is probably accompanied by internal hydrolysis of the peptide bonds (X + Y)-mer + H_2O = X-mer + Y-mer, as a result of which equilibrium is established under the given conditions and n remains constant. Having measured the partial pressure of water in the system, Meggy calculated that for this reaction ΔH = 3.3 kcal mole^{-1} and ΔS = = 5.9 kcal mole^{-1} deg^{-1}.

Attempts at polycondensation of other amino-acids were unsuccessful. α-Aminoisobutyric acid does not change on heating with a small amount of hydrochloric acid at 140° for 24 h. At 220–240° traces of a sublimate were observed, which Meggy believes to be 3,3,6,6-tetramethyl-2,5-dioxopiperazine. Prolonged heating at this temperature resulted in appreciable pyrolysis of the initial amino-acid. Under similar conditions DL-phenylalanine decomposes partially with the formation of resinous products; L- and DL-alanine is converted into dark viscous products which are completely soluble in water. Thus the majority of α-amino-acids do not form homopolypeptides on heating. The ease with which glycine undergoes polycondensation is probably due to the absence of a side-chain capable of causing steric hindrance.

Fox and Middlebrook [20-22] suggested that the conditions of the thermal polycondensation of amino-acids are very similar to the conditions obtaining in the formation of protein species on earth and showed that amino-acids which fail to yield peptides when heated in a pure form are partially converted into linear polypeptides when they are heat-treated in a mixture with other amino-acids. Further study led to a number of interesting findings and the observation of a number of interesting phenomena. Thus when equimolar mixtures of the DL-forms of aspartic acid, leucine, valine, and phenylalanine are heated at 200° for 0.5–3 h, in addition to appreciable quantities of acetone-soluble tarry products, polymers were obtained whose hydrolysate contained the initial amino-acids [23,24]. On the basis of the results obtained for the polycondensation of various pairs of amino--acid in different combinations, Fox and Harada concluded that copolymeric peptides are obtained by the thermal method only when the reaction mixture contains either glutamic or aspartic acid [25]. Table 1 presents the results of biuret tests of the products of the joint thermal condensation of glutamic acid with other α-amino-acids. Usually [25] L- or DL-glutamic acid was heated for 0.5–2 h with various amino-acids in equimolar proportions at 160–190°. The

TABLE 1.

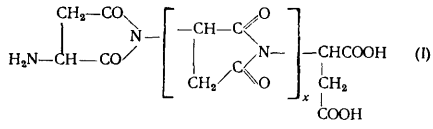
Reactants	Results of biuret test	Reactants	Results of biuret test
Glutamic acid + glycine	+	Pyroglutamic acid + lysine hydrochloride	+ (intense)
Glutamic acid + glycyl-glycine	+	Pyroglutamic acid + + leucine + glycine	+
Glutamic acid + 2,5-dioxo-piperazine	+	Pyroglutamic acid + + proline + glycine	+
Pyroglutamic acid + + glycine	+	Pyroglutamic acid + + aspartic acid + glycine	+
Pyroglutamic acid + 2,5--dioxopiperazine	0	Pyroglutamic acid + lysine hydrochloride + glycine	+
Pyroglutamic acid + 2,5--dioxopiperazine + water	+	Pyroglutamic acid + lysine hydrochloride + cystine	+
Glutamic acid + aspartic acid	+	Pyroglutamic acid + lysine hydrochloride + cystine + + glycine	+
Pyroglutamic acid + + aspartic acid	+	2,5-Dioxopiperazine + + glycylglycine	0
Glutamic acid + asparagine	+	2,5-Dioxopiperazine + + glycine + phenylalanine	0
Pyroglutamic acid + alanine	+	Glycine + aspartic acid	+ (weak)
Pyroglutamic acid + valine	+	Glutamine + aspartic acid	+
Pyroglutamic acid + leucine	+	Glutamine + asparagine	+ (weak)
Pyroglutamic acid + phenyl-alanine	+		
Pyroglutamic acid + serine	at 160° the product darkens before the test		

molecular weight of the copolymers with glycine reached 10 000–20 000.

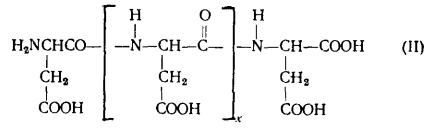
During thermal copolycondensation glutamic acid is converted into pyroglutamic acid[26] which melts in the presence of other substances at 170° and probably behaves as a solvent for the reaction mixture thus favouring a more rapid and complete reaction. Furthermore, a relative estimate of the yields of the products showed that pyroglutamic acid is more reactive than glutamic acid. It is also extremely probable that glutamic and pyroglutamic acids can behave as "acid" catalysts for amino-acid copolycondensation, since it is known that the formation of the amide bond is catalysed by protons, in particular in the presence of traces of water. However, the above workers did not present any evidence for this hypothesis.

Heating of aspartic acid does not result in the formation of any liquid products at temperatures below 200° but the acid is also capable of forming copolymers, which in contrast to the copolymers with glutamic acid, contain a smaller proportion of neutral and basic acids. A striking property of aspartic acid is that it is capable of homopolymerisation on heating above 100°. This interesting property attracted attention long ago and has been the subject of numerous studies.

It has been shown[27,28] that polyaspartic acid is a polyimide (I), which firmly retains water (approximately one molecule of water per imide bond); the latter can only be removed by thorough drying at 200° in a vacuum. The polyimide polyhydrate is converted into polyaspartic acid with the structure of a true polypeptide (II) on brief heating in dilute sodium hydroxide solution. The mean molecular weight of polyaspartic acid, which is approximately 110 000, was determined by end-group analysis and from the rate of sedimentation in the ultracentrifuge.

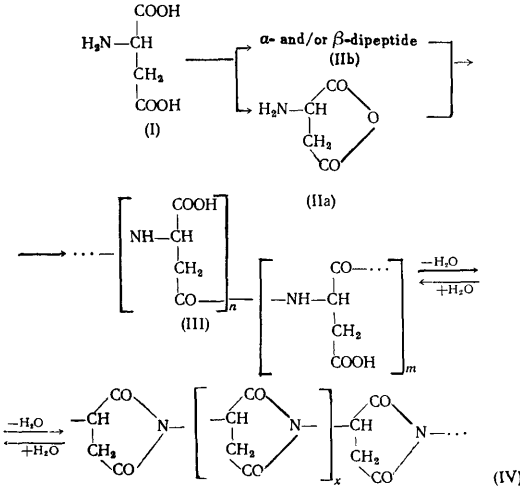


(I)



(II)

Earlier, Kovacs, Könyves, and coworkers[29-34], who studied the mechanism of the thermal polycondensation of aspartic acid and the structure of the resulting polymeric products, arrived at a similar conclusion. The polycondensation of aspartic acid was carried out either by heating in a vacuum (0.1 mmHg) at 200° or by boiling a suspension of aspartic acid in tetralin which made it possible to remove the water, evolved during the condensation, by azeotropic distillation. The authors found that thermal polycondensation of aspartic acid (I) yields the so-called "anhydropolyaspartic acid" (IV):



(IV)

Using the Hofmann degradation, the authors determined the ratio of the α- and β-links in the intermediate $\alpha\beta$-polyaspartic acid (III), which proved to be 1 : 1.3. The molecular weights of different specimens of substance (III) calculated by Van Slyke's method were in the range 6000–12 000.

Anhydropolyaspartic acid, prepared by different methods, had identical properties: it did not dissolve in water or a dilute solution of sodium bicarbonate, and on partial hydrolysis (0.1 *N* NaOH) it was converted into $\alpha\beta$-polyaspartic acid. In addition, study of the mechanism of the initial stage of the condensation showed that heating of acetylaspartic acid (145–200°) also yields anhydropolyaspartic acid (IV) instead of the expected monomeric anhydride.

When mixtures of aspartic and glutamic acids in various proportions were heated at 170–180°, polymers of fairly high molecular weight, with a polyimide structure according to infrared spectroscopic data, were obtained[35]. As in the case of the homopolymer of aspartic acid, the imide form of the copolymer is converted into the polypeptide on treatment with a hot solution of sodium hydroxide or boiling water. If the polypeptide thus isolated is heated, it is reconverted into the imide form, identical with the initial polymer. The authors propose the following mechanism for the mutual interconversions of the various forms of polyaspartic acid and the products of the thermal copolycondensation of glutamic and aspartic acids.

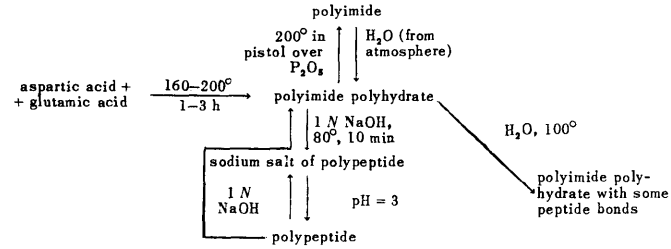


Table 2 presents data on the effect of temperature on the copolycondensation of L-aspartic and L-glutamic acids [35]. It is evident that the mean molecular weight and the yield appreciably increase with temperature. The dependence of the yield of the polymer on the proportions of the initial acid is given in Fig. 1, which shows that the optimum yield of the polypeptides is achieved when the aspartic : glutamic acid ratio is (3–4) : 1.

It was found that the glutamic acid content of the copolymer in the *N*-terminal position is 2–2.5 times greater than its total content in the polypeptide (Table 3).

On the basis of these data, Fox and Harada put forward the hypothesis [35-39] that on copolycondensation amino-acids "spontaneously" take up a specified ordered arrangement in the polymeric chain.

The results of the polycondensation of glutamic and aspartic acids with one another and with other α-amino--acids show that all 18 amino-acids which are present in natural proteins can take part in polycondensation simultaneously. As a result of such synthesis, Fox and Harada obtained a number of protein-like substances which they called "proteinoids"; the qualitative composition of their hydrolysates was the same as that of most natural proteins [36-38]. "Proteinoids" are fairly readily obtained only in the presence of a large excess of aspartic and glutamic acids. When they are present in equimolar proportions with other amino-acids, side-products are formed (for example 2,5-dioxopiperazines) in addition to peptides, and the isolation of the peptides becomes difficult.

The proteinoids were prepared by heating (2 h at 170–180°) two parts of glutamic acid, two parts of aspartic acid, and one part of an equimolar mixture of the remaining amino-acids. Apart from an excess of the acid amino--acids, the success of the reaction requires the removal of water from the reaction vessel, which is achieved by heating the reactants above 100°. Glutamic acid may be replaced by glutamine. The reaction is accelerated by orthophosphoric acid, whose effect on the copolycondensation of α-amino-acids was studied using as an example the copolycondensation of aspartic acid with glutamic acid and of aspartic acid with glycine, which takes place readily [39]. Phosphoric acid increases the yield but has almost no effect on the molecular weight of the polypeptides. On more prolonged heating, the amount of product which does not dialyse through the cellophane membrane increases, which is probably due to an increase in the yield of the high-molecular-weight fraction of the polymer. The formation of non-dialysable polymer after heating for 1 h begins at a temperature as low as 150°. In the absence of phosphoric acid comparable results are obtained only at 170°. Table 4 presents data on the effect of temperature on the copolycondensation.

In the presence of phosphoric acid the *N*-terminal composition of the copolymer changes little and the aspartic acid content of the copolymer rapidly increases with increase of the aspartic acid : glutamic acid ratio in the initial mixture. The difference between the aspartic acid : glutamic acid ratios calculated from the total and *N*-terminal compositions is in this case even greater. It follows from this that phosphoric acid favours the self-regulation of the disposition of the amino-acid residues in the polypeptide chain. The relative content of phosphoric acid in the initial reaction mixture has an appreciable effect on the rate of condensation (Fig. 2). The authors believe that phosphoric acid can behave in this reaction both as a solvent and as an acid catalyst and can also facilitate the removal of water from the reaction sphere and the formation of intermediate active compounds [40].

All the proteinoid fractions gave positive biuret, xanthoproteic, and other qualitative tests for protein. In the cold proteinoids do not react with ninhydrin. However, in hot solutions they give the colour characteristic of this test (clearly as a result of the partial removal of amino-acids)

TABLE 2. Copolycondensation of L-aspartic (0.01 M) and L-glutamic (0.01 M) acids.

Temperature, °C	Yield of crude polymer, g	Yield of polymer after dialysis, g	Mean molecular weight of the copolymer
160	0.64	—	—
170	0.19	0.16	—
180	0.49	0.40	5 100
190	0.88	0.71	13 000
200	0.98	0.83	18 000

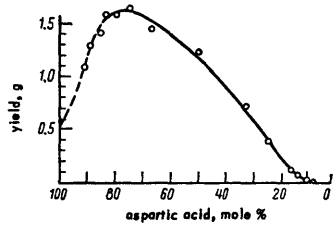


Fig. 1. Variation of the yield of the copolymers of aspartic and glutamic acids with the reactant ratio; the total amount of aspartic and glutamic acids in each determination was 0.025 mole; temperature 180°; duration of reaction 3.5 h; in the region corresponding to the continuous line the reaction mixture was present in a single phase.

TABLE 3.

Moles of aspartic acid in reaction mixture	Moles of glutamic acid in reaction mixture	Content of glutamic acid in the polymer as a whole (A), %	Content of glutamic acid in the *N*-terminal position (B), %	B/A
0.01	0.01	29	59	2.0
0.02	0.01	19	54	2.8
0.03	0.01	15	54	3.6

TABLE 4. Effect of temperature on the thermal copolycondensation of aspartic (0.02 M) and glutamic (0.01 M) acids in the presence of 0.8 ml of 85% orthophosphoric acid.

Temperature, °C	Yield of polymer before dialysis, g	Yield of polymer after dialysis, g	Aspartic acid : glutamic acid ratio in copolymer		Mean molecular weight	
130	1.15	0				
140	0.39	traces				
150	0.62	0.44				
160	2.00	1.61				
170	1.99	1.76	17	2.1	6700	0.01
180	2.27	2.09	18	1.5	11 800	0.018
190	2.28	2.13	11	1.6	12 300	0.024
200	2.30	2.14	12	1.0	33 600	0.020
210	2.33	2.18	9	1.1	25 600	0.014

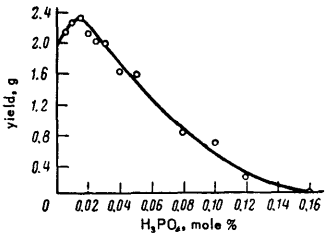


Fig. 2. Variation of the yield with the number of moles of 85% phosphoric acid in the copolymerisation of 0.02 mole of aspartic acid and 0.01 mole of glutamic acid.

but at a slower rate than natural proteins. The mean molecular weight of proteinoids is smaller than that of the copolymers of aspartic and glutamic acids obtained under the same conditions, amounting to about 4000–8000.

Infrared spectra show that proteinoids have a typical polypeptide structure. In addition, the spectra show absorption bands characteristic of imide bonds but the peaks characteristic of 2,5-dioxopiperazines are completely absent.

The solubility of proteinoids depends on the method of preparation. Copolymers synthesised in the usual way were nine-tenths insoluble in water but were soluble in salt solutions, for example in 0.1 *N* phosphate buffer at pH 7.9 or in 3.5% solution of sodium chloride. Like natural proteins, synthetic proteinoids can be salted out by the addition of a saturated solution of ammonium sulphate to the salt solution.

Proteinoids can replace the peptone in the broth culture of *Lactobacillus arabinosus*; although the growth rate of the bacteria in the broth based on the proteinoid is somewhat lower, the very fact of the growth of the microorganisms indicates that the links in the proteinoid are susceptible to attack by bacterial proteases.

Thus in many of their properties the products of the thermal copolycondensation of the mixture of 18 amino-acids closely resembles natural proteins. However, heating at 170–180° inevitably results in partial racemisation of the asymmetric centres in the amino-acids and, in contrast to the protein chain, the peptide chain of protenoids is built up of amino-acid residues of different configuration. As a result of this, it is impossible to obtain by this method polypeptides with the steric structure of protein molecules.

The available data are insufficient for a complete answer to the problem of the arrangement of the amino-acid residues in proteinoids — whether the arrangement is completely random or whether it is ordered in some specified way. The authors of the investigation believe that, since the *N*-terminal ratios of glutamic acid, aspartic acid, and neutral amino-acids appreciably differ from the ratios in the total composition and from the ratios of the components in the initial mixture (Tables 5 and 6) and since the *N*-terminal amino-acid composition varies with the temperature of the synthesis while the total composition remains constant, the thermal reaction of the mixture of amino-acids cannot be regarded as a completely disordered process.

Krampitz[41-43] obtained several proteinoids by heating a mixture of amino-acids in sealed tubes in an atmosphere of nitrogen (3 h at 170°). Proteinoid (I) was synthesised from two parts of aspartic acid, two parts of glutamic acid, and one part of an equimolar mixture of other amino-acids, namely: DL-alanine, DL-isoleucine, DL-thereonine, DL-valine, DL-methionine, DL-phenylalanine, DL-serine, DL-tryptophan, L-arginine, L-histidine, L-proline, L-leucine, L-tyrosine, glycine, and lysine monohydrochloride. Proteinoid (II) contained four parts of aspartic acid, four parts of glutamic acid, and one part of the above equimolar mixture of amino-acids. After purification the copolymers give the following positive reactions: biuret, xanthoproteic, Hopkins–Cole–Adamkewicz, Salkowski, and the reaction with Azocarmine B. The *N*-terminal positions in both proteinoids contain mainly aspartic and glutamic acid residues, the contents of neutral and basic acids being only slight. It was found that the hydrolysis of copolymers (I) and (II) by a number of proteolytic enzymes, such as pepsin, trypsin, erepsin, and papain proceeds to different extents. Similar results were obtained by Fox and Harada, who studied the effect of chymotrypsin and pepsin on two proteinoid specimens[37]. Fig. 3 presents the results of the proteolysis of the synthetic products and casein. It is evident that casein is hydrolysed more

TABLE 5. Effect of temperature on the amino-acid composition of proteinoids for two amino-acid ratios.

Temperature, °C	Aspartic acid, %	Glutamic acid, %	Neutral amino-acid, %
2:2:1 ratio			
160	66	13	22
170	65	12	23
180	63	13	24
190	56	14	29
1:1:1 ratio			
160	56	14	30
170	55	13	32
180	55	14	31
190	46	19	35

TABLE 6. Effect of temperature on the *N*-terminal amino-acid composition of proteinoids for two amino-acid ratios.

Temperature, °C	Aspartic acid N : amino-acid ΣN, %	Glutamic acid N : amino-acid ΣN, %	Neutral acid N : amino-acid ΣN, %
2:2:1 ratio			
160	7	57	36
170	6	46	48
180	11	37	53
190	8	13	80
1:1:1 ratio			
160	8	42	50
170	7	30	63
180	10	20	71
190	9	10	81

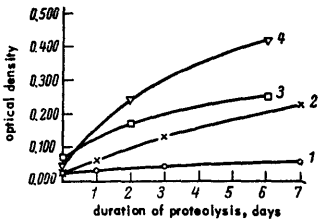


Fig. 3. Proteolysis of proteinoids with pepsin and chymotrypsin:
1) proteolysis of 2 : 2 : 1-proteinoid with chymotrypsin; 2) proteolysis of casein with chymotrypsin; 3) proteolysis of 1 : 1 : 1-proteinoid with pepsin; 4) proteolysis of casein with pepsin.

completely by the enzymes than the proteinoid, because proteases act only upon the links between L-amino-acid residues. Pepsin is particularly active in the presence of acid amino-acid residues and for this reason proteinoids are more susceptible to "attack" by pepsin than by other enzymes.

Like dicarboxylic amino-acids, free DL-lysine is converted at 150–170° into the liquid lactam and at 180–230° it undergoes homopolymerisation[44]. Lysine also readily condenses with glycine, alanine, and other neutral amino--acids, glutamic and aspartic acids, caprolactam, and succinic, terephthalic, and other acids. The infrared spectra of the lysine homopolymer and the copolymers of lysine with neutral amino-acids and with glutamic acid show that these are typical polypeptides. Lysine monohydrochloride is incapable of homopolycondensation but it readily undergoes copolycondensation with glutamic acid and other amino-acids in the presence of orthophosphoric acid.

The lysine homopolymer is soluble in water but does not dialyse through a cellophane membrane. The copolymers of lysine with glutamic acid and with glycine are gel-like substances while its copolymer with aspartic acid is a spongy solid mass. The amorphous copolymers of lysine with glutamic and with aspartic acids swell in alkalis and acids but do not dissolve in them. This is probably because the copolymers are highly branched and probably possess to some extent also a three-dimensional structure.

In 1961 Fox et al.[45] reported the thermal copolymerisation of all 18 α-amino-acids in the presence in the reaction mixture of acid or basic components. One of the copolymers was obtained on heating the mixture of amino-acids with an excess of aspartic acid and another, under similar conditions, with an excess of lysine. In many of their properties both copolymers closely resemble proteins. The first proteinoid readily dissolved in water, dilute sodium bicarbonate solution, and phosphate buffer, had a molecular weight of the order of 3000–9000, and an isoelectric point close to 2.8. The infrared spectrum shows that the copolymer contains a considerable number of imide links which disappear when the proteinoid is treated with alkali, its spectrum then becoming typical of a polypeptide structure. Copolymer (II) was an elastic rubberlike substance soluble in mineral acids and only partially soluble in water (10%). Its molecular weight exceeded 25 000, its isoelectric point was of the order of 8.4, and it had the additional interesting feature that it could form films.

Soon after the start of the investigation of the thermal synthesis of peptides, Fox et al.[23] observed by chromatography that the hydrolysis mixtures contain a greater number of amino-acids than were taken initially in the reaction. This is probably because under the conditions of the thermal polycondensation the initial reactants undergo various chemical changes with the formation of new amino-acids and other products. It was shown, for example, that aspartic acid is converted into α- and β-alanine, and on heating with urea into ureidosuccinic acid, an intermediate in the synthesis of many pyrimidine bases. It is also interesting that spots characteristic of alanine and aspartic acid were found on chromatograms of the hydrolysis products from polymers obtained on heating ammonium fumarate and maleate.

Meggy[46] described the synthesis of polymers on heating glycine in water at 140–180° in an autoclave. The yield of products varied with temperature and the reactant ratio. The reaction is catalysed by hydrochloric acid, nitric acid, sulphuric acid, phosphoric acid, ammonium hydroxide, sodium hydroxide, and potassium hydroxide.

Bahadur[47–49] studied the photosynthesis of α-amino-acids and peptides in aqueous media. The reaction of electric light on a sterilised mixture of paraformaldehyde, water, and colloidal molybdenum oxide resulted in the formation of alanine and glycine together with a small amount of valine, histidine, glutamic acid, and ornithine, the nitrogen required for the synthesis being "fixed" from the atmosphere. Illumination of aqueous solutions of glycine in the presence of sucrose leads to the formation of oligomeric peptides, in particular glycylalanine, glycylnorleucine, and glycylglycine. When sterilised solutions of glycine and aspartic acid were illuminated with a 1000 W electric lamp for 400 h in the presence of benzoyl peroxide as the photocatalyst and sucrose as an additional energy source, new amino-acids and peptides were formed. A control experiment in the dark gave a negative result.

Ellenbogen[50] published an interesting paper on the synthesis of polypeptides on illumination with a mercury or a hydrogen lamp of aqueous solutions of ammonium salts through which a mixture of hydrocarbons (from methane to pentane) was passed continuously. The catalysts were certain salts of sodium, potassium, calcium, and a number of heavy metal oxides. The amino-acid composition of the resulting polypeptides varied with the composition of the reaction mixture and the conditions of synthesis.

Apart from early work[51–54] in which it was shown that thermal polycondensation of glycylglycine and glycylalanine usually yields 2,5-dioxopiperazines, there is no information in the literature on the polycondensation of di- and tri--peptides, probably because no significant results were obtained in such attempts.

2. Synthesis of Polypeptides Using Condensing Agents

Synthetic polypeptides frequently have a relatively low molecular weight; it may be increased by means of condensing agents which are widely used in experiments on stepwise synthesis of peptides with a known amino-acid sequence. Such condensing agents are *NN'*-dicyclohexylcarbodiimide[55], tetraethyl pyrophosphite[56], phosphorus pentoxide[57], etc. Furuyama et al.[58] used the relatively readily available bis-*o*-phenylene pyrophosphite as a condensing agent. After polycondensation of L-leucylglycylglycine in dry pyridine (for 6 h at 100°), two polypeptide

TABLE 7.

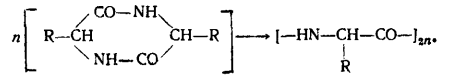
Initial peptide	M	Final polypeptide	
		solvent	M
Poly(γ-benzyl L-glutamate)	200 000	dioxan	950 000
Poly(γ-benzyl L-glutamate)	225 000	chloroform	1 000 000
Poly-ε-benzyloxycarbonyl-L-lysine	350 000	chloroform	840 000
Poly-L-proline	10 000	water	32 000
Poly(γ-benzyl L-glutamate) + poly-ε-benzyloxycarbonyl-L-lysine(1 : 1)	200 000 + 300 000	chloroform	6 000 000

fractions with molecular weights (M) of 3220 and 1730 were isolated. The polypeptides dissolved in 80% aqueous phenol and 85% formic acid.

When NN'-dicyclohexylcarbodiimide is used for the polycondensation of oligomeric peptides with free carboxy- and amino-groups, polymers with high molecular weight are obtained (Table 7)[59]. The polycondensation was carried out at room temperature in different organic solvents. The authors of these studies believe that the condensation involves three terminal NH_2 and COOH groups. In those cases where the polypeptide has terminal amino- and carbamoyl groups, no increase in molecular weight is observed. The method can also be used to prepare high-molecular-weight polypeptides, including water-soluble peptides, whose synthesis by other methods presents considerable difficulties. In the presence of free carboxy- and amino-groups at the end of the peptide chain, this method can yield block copolymers and branched-chain polypeptides.

3. Polymerisation of 2,5-Dioxopiperazine Derivatives

It has been shown in a number of investigations[60-63] that 2,5-dioxopiperazine is thermodynamically less stable than polyglycine and is relatively readily converted into the polypeptide under various conditions. By analogy with glycolide and lactide, which polymerise into linear polyesters, it might have been expected that substituted 2,5-dioxopiperazine would also polymerise into polypeptides by the following reaction:

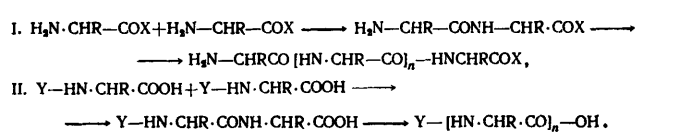


However, there have been only a few studies on the polymerisation of 3,6-substituted 2,5-dioxopiperazines. Goto[64] achieved the copolymerisation of 3,6-diisobutyl-2,5-dioxopiperazine with 2-oxopyrrolidine-5-carboxylic acid by heating an equimolar mixture of the starting materials (200°, 36 h) in glycerol. The copolymer thus isolated (yield 70%) gave a positive biuret reaction. The author believes that the product is the polypeptide, which he called a "protein analogue". Fox and Harada[25] mentioned the possibility of the copolymerisation of glutamic acid with 2,5-dioxopiperazine. However, they established that, when 2,5-dioxopiperazine and 2-oxopyrrolidine-5-carboxylic acid are heated together under anhydrous conditions, no polypeptide is formed and only after the addition of a certain amount of water to the mixture does the copolymer give a positive biuret test.

Attempts to prepare polypeptides by hydrolytic polymerisation of 1-ethyl-1,4-diphenyl- and 3,6-dimethyl-2,5-dioxopiperazines were unsuccessful. Hall[65] failed to obtain positive results in the anionic polymerisation of 1,4-dimethyl-2,5-dioxopiperazine in the presence of metallic sodium, sodium hydride, and acetylcaprolactam.

III. SYNTHESIS OF POLYPEPTIDES FROM α-AMINO-ACID DERIVATIVES WITH MODIFIED AMINO- OR CARBOXY-GROUPS

The low tendency of free α-amino-acids and dipeptides towards polycondensation and their marked tendency to undergo cyclisation precludes their use for the synthesis of high-molecular-weight peptides under conditions excluding the possibility of racemisation of asymmetric centres. To facilitate the formation of the peptide bond, it is necessary to activate the carboxy- or the amino-group of the acid. An enhanced reactivity of amino-acids is usually achieved[4,5] by converting them into various O- or N-derivatives in which the link between the substituent and the amino-acid residue is readily susceptible to aminolysis in the case of O-derivatives or to acylation in the case of N-derivatives.



Usually such derivatives are esters and thioesters of amino-acids and peptides, amides, acid chlorides, azides, and also N-benzyloxycarbonyl-, N-phenylthiocarbonyl-, N-nitrophenoxycarbonyl-, and other derivatives of amino-acids.

1. Polycondensation of α-Amino-Acid Amides and Nitriles

Kovacs and Nagy[66] described the separation of polyaspartic acid by boiling a 5–10% aqueous solution of L-asparagine. Part of the asparagine hydrolyses to aspartic acid but most of it is converted into an amorphous mixture of oligo- and poly-peptides, among which, in particular, L-asparagylaspartic acid was identified. The mean molecular weight of the polymeric products isolated was about 500, the yield being 63%. If the reaction is carried out at 70–75°, the yield falls to 18%. Polymers of high molecular weight were obtained by dialysis. The mean molecular weight of various preparations after dialysis varied from 1700 to 3000. Like αβ-polyaspartic acid, these polypeptides readily dissolve in water and give a positive biuret reaction; their infrared spectra are identical with those of polyaspartic acid.

Guidry[67,68] studied the polycondensation of glycine amide in aqueous ammonia solution. The reaction was carried out by heating glycine amide (or its hydrochloride) with half its weight of ammonium hydroxide solution at 100° for several hours. The product was purified by dialysis and then lyophilised. Table 8 presents data on the yield and mean degree of polymerisation.

The authors quote preliminary kinetic data on the polycondensation of glycine amide, which are presented in Fig. 4. The graph shows that the initial rate of reaction is fairly high, so that polycondensation can successfully compete with hydrolytic and aminolytic side-reactions. The polycondensation products were white or slightly coloured powders, insoluble in water but soluble in concentrated lithium bromide solutions; they gave the biuret reaction

TABLE 8.

Composition of reaction mixture	Mean degree of polymerisation	Molar yield of polyglycine, %
2 g of glycine amide hydrochloride + + 1 ml 2 *N* NH_4OH	40	16.5
2 g glycine amide hydrochloride + + 1 ml 8 *N* NH_4OH	29	74.8
Glycine amide (not recrystallised)	33	27.4
Glycine amide (recrystallised)	34	75.0

Note. The specimens were heated in closed tubes at 100° for 20 h and more. The degree of polymerisation of the first two substances was determined by formol titration by the Sorensen method in lithium bromide solution, and that of the other two by the Van Slyke method.

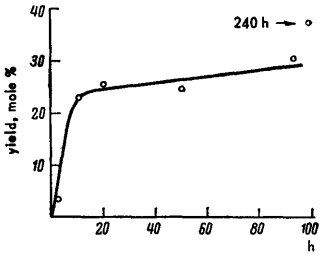


Fig. 4. The rate of formation of polyglycine from glycine amide on heating the latter in ammonium hydroxide solution at 100°.

and were completely hydrolysed to glycine in boiling acid. The infrared spectra of the polymers showed two broad absorption bands characteristic of the NH and CO bending vibrations (1534–1562 and 1634–1648 cm^{-1} respectively), which means that the polymers contain predominantly bonds corresponding to the α- and β-forms of polyglycine. This is also indicated by the presence of absorption bands in the region of 1015 cm^{-1} (α) and 1028 cm^{-1} (β). At a lower reaction temperature (60°) the polyglycine was obtained in 20% yield.

Copolycondensation of glycine amide with the amides of other amino-acids does not give appreciable yields of polymeric products, but the residues of most of the amino-acids whose amides were used as starting materials were detected in the copolymers.

On heating aminoacetonitrile[67] in aqueous ammonia, a black polymer was obtained, which however could not be characterised. Heat treatment of methyleneaminoacetonitrile in the presence of ammonium sulphate leads to the formation of a non-dialysable product consisting mainly of glycine and probably cysteine residues. All the peptides were characterised by the usual analytical methods and had relatively low molecular weights of the order of 570–930.

Mach put forward the hypothesis[69] that the primary synthesis of amino-acids and proteins took place in three stages. Initially aminoacetonitrile was formed from the simple compounds HCN, HCOOH, and NH_3 and this then underwent polycondensation into polyglycine. The reaction of the polyglycine with aldehydes and unsaturated compounds in the third stage resulted in the synthesis of polypeptides. To confirm this hypothesis, the author heated one part of aminoacetonitrile with kaolin (20 parts) at 130° for 5 h. Chromatographic analysis revealed the presence in the reaction products of glycylglycine, diglycylglycine, and polyglycine. After treatment of the polyglycine with formaldehyde in the presence of basic catalysts, serine was detected in the hydrolysis mixture.

Losse and Anders[70] studied the polymerisation of α-aminopropionitrile on mineral carriers. They regarded this reaction as one of the possible ways in which protein is synthesised in nature. One part of α-aminopropionitrile was heated with two parts of a mineral adsorbent (Fuller's earth) for 50 h at 80° and 5 h at 130°. The initial nitrile was then converted to the extent of 60% into polyamidine, the hydrolysis of which yielded polyalanine with a number-average molecular weight of the order of 20 000.

$$n\,H_2N{-}\underset{\displaystyle CH_3}{\underset{|}{CH}}{-}C{\equiv}N \longrightarrow (-HN{-}CH(CH_3){-}\underset{\displaystyle NH}{\underset{\|}{C}}{-})_n \xrightarrow[-n NH_3]{+n H_2O} (-HN{-}CH(CH_3)\,CO{-})_n.$$

The polypeptide was isolated, characterised by the usual analytical methods, and compared with the polymer obtained by the synthesis from *N*-carboxyalanine anhydride. The properties of both polypeptides proved to be identical. It is interesting that, when α-aminopropionitrile is heated in the absence of a mineral carrier, there is virtually no polymerisation, $\alpha\alpha'$-iminodipropionitrile $HN[CH(CH_3)CN]_2$ being formed.

2. Polycondensation of α-Amino-Acid Esters

The alkoxy-group in amino-esters is fairly active and readily undergoes aminolysis, particularly in the presence of acid catalysts[71]:

$$H_2NCHRCOOR' + H_2NCHRCOOR' \xrightarrow{H^+} H_2NCHRCOHNCHRCOOR'.$$

Thus polycondensation of amino-acid esters would appear to be a convenient way of synthesising linear polypeptides; however, the main reaction in the polycondensation is frequently the cyclisation of the ester of the dipeptide formed into 3,6-disubstituted 2,5-dioxopiperazines[72,73]. Thus the methyl esters of glycine and DL-alanine gave 53 and 96% respectively of 2,5-dioxopiperazines on their polycondensation in the presence of 2% CO_2 [74] and SiO_2.[75]

The characteristic features of the polycondensation of glycine and DL-alanine esters in the presence of carbon dioxide and ester carbamates have been investigated[76,77]. According to the authors, the first stage in the reaction is rapid formation of the carbamate of the amino-acid ester:

$$CO_2 + 2H_2NCHRCOOR' \longrightarrow R'OOCCHR\overset{+}{N}H_3\cdot\overset{-}{O}OCNHCHRCOOR'.$$

The reaction is strongly exothermic and in the case of the ethyl ester of glycine terminates after 2–3 s with absorption of one mole of carbon dioxide for two moles of the ester[78]. The resulting carbamate is fairly stable, decomposing into the initial compound at about 60°.

In the symmetric carbamate one ester group is in the activated state and readily undergoes aminolysis. Thus in the polycondensation of amino-esters the growth of peptide chains proceeds as a result of the reaction of the symmetric carbamate with the amino-groups of the peptides or (in the

case of the initiation of a new chain) with the amino-group of the amino-acid ester with the formation of an asymmetric carbamate [77,79]:

$$R'OOCCHR\overset{+}{N}H_3\cdot\overset{-}{O}OCNHCHRCOOR'+H-[HNCHRCO]_x-OR'\xrightarrow{-R'OH}$$

$$\longrightarrow R'OOCCHR\overset{+}{N}H_3-\overset{-}{O}OCNHCHRCO-[HNCHRCO]_x-OR'\longrightarrow$$

$$\longrightarrow R'OOCCHRNH_2+CO_2+H-[HNCHRCO]_{x+1}-OR'.$$

Because of its instability [80], the asymmetric carbamate breaks down with the regeneration of carbon dioxide and the formation of a peptide containing one amino-acid residue more than the initial peptide or, what is more probable, the carbamate reacts with a new molecule of the monomer with the formation of the symmetric carbamate and the free peptide [81]:

$$R'OOCCHR\overset{+}{N}H_3\cdot\overset{-}{O}OCHNCHRCO[HNCHRCO]_xOR'+H_2NCHRCOOR'\longrightarrow$$

$$\longrightarrow R'OOCCHR\overset{+}{N}H_3\,\overset{-}{O}OCHNCHRCOOR'+H[HNCHRCO]_{x+1}OR'.$$

The increase of the reactivity of the ester group in the symmetric carbamate of the amino-acid ester, as a result of the influence of the carbamate group, was confirmed by the study of the polycondensation of the ethyl ester of glycylglycine. In this case the increased distance between the carbamate and ester groups results in a sharp decrease of their mutual influence, which is reflected in an almost complete absence of the catalytic effect of carbon dioxide on the polycondensation of the glycylglycine ester [82].

The polycondensation process probably proceeds in steps, its rate increasing with the concentration of the initiator (Fig. 5).

The slowest stage in the polycondensation of ethyl glycinate is the formation of the ester of the dipeptide, which is relatively rapidly converted into the tripeptide and 2,5-dioxopiperazine. Figs. 6 and 7 show the variation of the products of the polycondensation of ethyl glycinate with the duration of the reaction. At the beginning of the process almost half the solid products consist of 2,5-dioxopiperazine, whose relative amount diminishes as the process approaches completion. The amount of tripeptide reaches a maximum and then gradually falls as a result of its rapid conversion into the polymer. The process is autocatalytic and, as the peptides accumulate and their length increases, there is an increase of the relative rate of consumption of the monomer by virtue of the polypeptide chain growth.

Determination of the rate of aminolysis of *p*-nitrophenyl esters of benzyloxycarbonyl derivatives of glycine and its

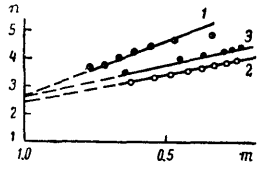


Fig. 5. Variation of the mean degree of polymerisation n of the initial fraction with the consumption of the monomer m for different initial molar ratios ξ_0 of carbon dioxide and the monomer: 1) $\xi_0 = 0.02$; 2) $\xi_0 = 0.04$; 3) $\xi_0 = 0.08$.

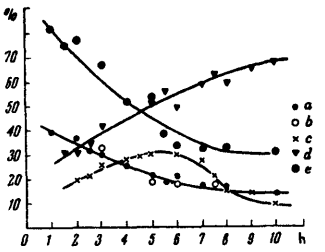


Fig. 6. Composition of the polycondensation products of ethyl glycinate (%): *a*) 2,5-dioxopiperazine, determined from the increase in amino-nitrogen; *b*) 2,5-dioxopiperazine, determined by the "copper" method; *c*) ethyl ester of diglycylglycine; *d*) tetrapeptide fraction; *e*) free ethyl glycinate.

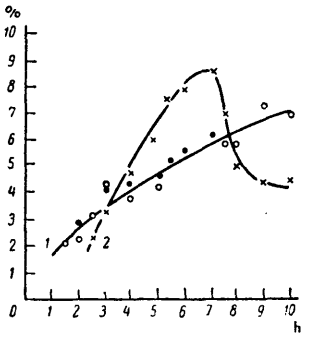


Fig. 7. The content of 2,5-dioxopiperazine and the ethyl ester of diglycylglycine (as a percentage of the initial monomer sample): 1) 2,5-dioxopiperazine; 2) ethyl ester of diglycylglycine.

di- and tri-peptides by ethyl glycinate and also the esters of the di- and tri-peptides indicates an increase in the rate of reaction of the amino- and ester groups with increase in chain length, particularly on going from the monomer to the dipeptides, which is consistent with the idea that polycondensation of amino-acid esters is autocatalytic [86].

In the absence of carbon dioxide the polycondensation of ethyl glycinate is very slow and takes place in two stages. The rate of the first stage is very low, but subsequently it increases (Fig. 8). In the second stage the monomer is used up much more rapidly. Addition of the polycondensation mixture accelerates the reaction, which still takes place in two stages, but the first stage is much shorter. An even greater shortening of the first stage occurs on addition of 20 mole % of the methyl ester of alanylglycylglycine, the rate of the second stage remaining almost unchanged, as in the case where the polycondensation mixture was added [87]. It was found that the addition of ethanol has a similar accelerating effect [88] on the polycondensation, which the authors explained by the catalytic action of the alcohol on the aminolysis reaction.

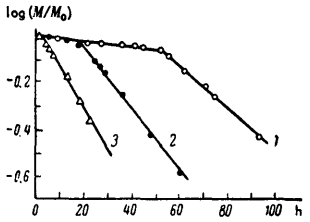


Fig. 8. Polycondensation of ethyl glycinate: 1) without additives; 2) in the presence of the polycondensate; 3) in the presence of the tripeptide ester.

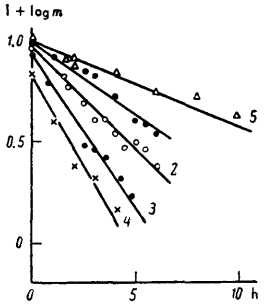


Fig. 9. Kinetics of the monomer consumption (m) in the polycondensation of ethyl glycinate; initial concentration of the initiator (ξ_0): 1) 0.02; 2) 0.04; 3) 0.08; 4) 0.16; 5) 0.01; the values of $m_0 - 1 - 2\xi_0$ are plotted along the ordinate.

Extrapolation of the data obtained in the polycondensation of ethyl glycinate with carbon dioxide (Fig. 9) shows that the true relative concentration of the monomer $(M_2 - \Delta M_0)/M_0 = m$, where ΔM_0 is the amount of monomer combined with carbon dioxide, is less than unity at zero time and decreases with increase in the concentration of the initiator. This confirms that, at the beginning of the reaction, part of the monomer $(1 - m_0)$ is rapidly combined and only then does the second stage of the reaction, characterised by first-order consumption of the monomer, begin.

Study of the kinetics of the polycondensation of ethyl glycinate in the presence of its carbamate [89,90] and carbon dioxide [87] showed that the nature of the process does not change when the method of initiation of the polycondensation is modified and confirmed that the carbamate of the amino-acid ester indeed catalyses the polycondensation. At all the catalyst concentrations investigated, the monomer is consumed by a first-order reaction. Comparison of the rate constants for the consumption of the monomer when the polycondensation of ethyl glycinate is initiated with the carbamate and carbon dioxide shows that the reaction kinetics in the two cases are the same (Fig. 10).

The nature of the polycondensation of the amino-esters does not change in the presence of the anhydrides of the N-carboxy-derivatives of the acids [91-93]. As in the polycondensation initiated with carbon dioxide, the reaction proceeds in two stages. In the polycondensation of ethyl

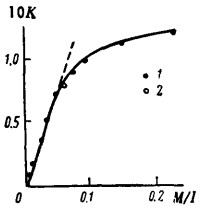
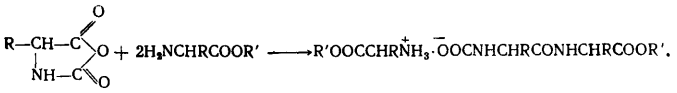


Fig. 10. Dependence of the rate constant for the consumption of the monomer (K, s^{-1} $mole^{-1}$) on the initial concentration of the initiator (weight fraction): 1) initiation with carbon dioxide; 2) initiation with carbamate.

glycinate and the anhydride of N-carboxyglycine it was found that in the first stage the ester is consumed very rapidly, which in the authors' view is due to the formation of the asymmetric carbamate:



The second stage, which is slower, proceeds by the carbamate mechanism with the formation of similar polycondensation products as in the case where the process is initiated with carbon dioxide or the carbamate of the amino-acid ester [93,94].

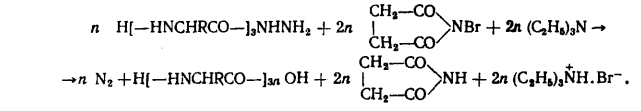
Metallic sodium and triphenylmethylsodium have also been described as catalysts for the polycondensation of amino-acid and peptide esters. Brenner and Rickenbacher [95] carried out the polycondensation of the methyl esters of DL-lysine and L-leucine (3–4 h at 5°) with a suspension of sodium (without additional solvent) and obtained in 60–70% yield polypeptides with a mean degree of polymerisation of 7 and 10 respectively. Polycondensation under these conditions of a mixture of the esters of L-leucine, DL-lysine, and DL-aspartic acid gave only a mixed polypeptide with a molecular weight of about 4000, which is difficultly soluble in water. The authors indicate that in the polycondensation of the methyl ester of L-leucine almost complete racemisation takes place. The authors explain the decrease of the yield of 2,5-dioxopiperazines when esters are subjected to polycondensation with metallic sodium by the adsorption of the amino-acid esters and the formation of polypeptides on the surface of the catalyst, while 2,5-dioxopiperazines form mainly in solution. In the polycondensation of esters in the presence of triphenylmethylsodium in ethereal solution the mean degree of polymerisation reaches 10–13. [96]

The polycondensation of the esters of oligopeptides has been considered in a relatively small number of studies. Thus Poroshin et al. state that when the methyl ester of L-prolyl-glycyl-L-proline is heated (15 h at 110° and 3×10^{-3} mmHg), a complex mixture of polypeptides is formed, which the authors did not analyse in detail [97]. De-Tar et al. [98] treated the hydrobromide of the p-nitrophenyl ester of β-methoxy-L-asparagylglycylglycine with the exact calculated amount of triethylamine in dimethylformamide or dimethylsulphoxide at room temperature and obtained a polypeptide with a molecular weight of about 12 000. It was shown that whether a linear polypeptide or cyclic compounds are formed is to a large extent determined by the concentration of the initial tripeptide ester. Thus treatment of a 15% solution of the

tripeptide ester resulted in a very small yield of polypeptides because of the formation of a cyclic hexapeptide. The authors used this method to prepare a number of polypeptides containing aspartic acid, phenylalanine, and glycine, or histidine and glycine, or phenylalanine and glycine. The synthesis of the last polypeptide from the hydrobromide of the *p*-nitrophenyl ester of phenylalanylglycine is particularly interesting, because it shows that linear polypeptides and not cyclic compounds are formed from the nitrophenyl esters of dipeptides.

Polypeptides of high molecular weight were prepared by the polycondensation of the thiophenyl $\alpha\gamma$-dimethyl ester of γ-L-glutamyl-L-glutamic acid, the mixture being slightly heated[99]. After purification the resulting polypeptide gave positive biuret and ninhydrin reactions. The authors demonstrated by paper chromatography that the polypeptide is polydisperse and contains high-molecular-weight fractions which remain at the starting point of the paper chromatogram after a 36 h run.

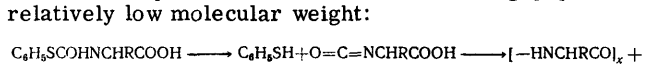
Among other work on the synthesis of polypeptides from tripeptide derivatives, that of Wolman et al.[100] is interesting. They oxidised the hydrazide of prolylglycylglycine with bromosuccinimide in dimethylformamide in the presence of triethylamine and obtained a polypeptide with a molecular weight of 1400. They suggested the following mechanism for the reaction:



The method is fairly simple and may be used when the initial tripeptide does not contain side-groups susceptible to oxidation by *N*-bromosuccinimide.

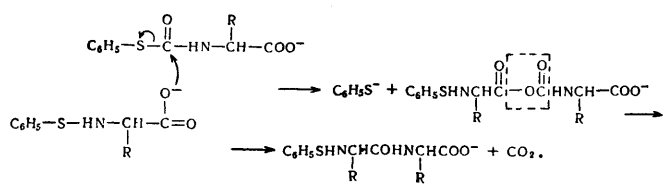
3. Polycondensation of *N*-Phenylthiocarbonyl Derivatives of α-Amino-Acids and Oligopeptides

Noguchi[101,102] showed that on heating *N*-phenylthiocarbonyl amino-acids to 150–160° thiophenol and carbon dioxide are split off with the formation of oligopeptides of relatively low molecular weight:



Apart from the polypeptides, the reaction products include 2,5-dioxopiperazine and other side-products. When the polycondensation is carried out in solution (frequently in the presence of pyridine), high-molecular-weight polypeptides with molecular weights up to more than 100 000 are formed.

Thermal polycondensation of *N*-phenylthiocarbonyl amino-acids can yield both homogeneous and mixed polypeptides of almost all nautral and basic amino-acids. The authors assumed the possibility that thiophenol may be split off as a result of the intermolecular reaction with subsequent removal of carbon dioxide from the anhydride and formation of the polypeptide chain:



Tables 9 and 10 list the polypeptides prepared by this method and some properties of the initial *N*-phenylthiocarbonyl amino-acids and polypeptides. The data in Tables 8 and 9 indicate the possibility of wide application of the method involving the polycondensation of *N*-phenylthiocarbonyl derivatives of amino-acids for the preparation of DL-polypeptides. When derivatives of dipeptides are used, the formation of polypeptides with a known sequence of amino-acid residues may be expected.

However, Weymouth[122] showed that this method has a number of disadvantages. When polyglycine is synthesised under the above conditions, considerable amounts of 2,5-dioxopiperazines (in excess of 20% of the total weight of products) are deposited on the walls of the vessel. Usually about 50% of the residue is soluble in water, and comparison of the viscosity of the solutions of these polymers with the viscosity of similar specimens of polyglycine, for which the end-groups have been determined, yields the molecular weight in the range 1000–2500. Noguchi and coworkers[104] found the molecular weight of polyglycine of similar viscosity to be 135 000.

Having checked Noguchi's data using the polycondensation of phenylthiocarbonylalanine and sarcosine, the author concludes that the polycondensation of *N*-phenylthiocarbonyl amino-acids involves the formation of polypeptides of relatively low molecular weight. Furthermore there is no proof that these polymers are purely linear polypeptides.

The polycondensation of other *N*-substituted amino-acids has been described, i.e. the polycondensation of *N*-benzyloxycarbonyl[123] and phenoxycarbonyl[124] derivatives, including those with different substituents in the phenyl ring. Ishizuka and Saito[125] established that the most suitable medium for the polycondensation of phenoxycarbonyl amino-acid derivatives is nitrobenzene. In this case the time of polycondensation is greatly reduced and the polypeptide has a higher molecular weight (Table 11).

Ishizuka[126–128] carried out the polycondensation of *N*-*o*-nitrophenoxycarbonyl derivatives of amino-acids and dipeptides by this method and obtained polyleucine (M = 14 000), poly-DL-phenylalanine (M = 12 000), polyglycine (M = 14 000), polyalanylglycine (M = 28 500), polyglycylalanine [a previous line is repeated at this point in the Russian text instead of the conclusion of the sentence (Ed. of Translation)].

The polycondensation of *N*-*p*-methylphenoxycarbonyl amino-acids, in solution in a mixture of benzene and toluene, in a sealed tube at 60° for 1200 h yielded polyglycine (M = 10 500), poly-DL-phenylalanine (M = 17 600), poly-L-leucine (M = 11 000), and poly-α-aminobutyric acid (M = 18 300). The molecular weights were determined osmometrically in dichloroacetic acid[129]. Polyleucine with an intrinsic viscosity of 0.28 was prepared by the polycondensation of *N*-phenoxycarbonyl-L-leucine in nitrobenzene at 125° for 20 h.[130]

Bruckner et al.[99] prepared a high-molecular-weight polypeptide from the $\alpha\gamma$-dimethyl ester of benzyloxycarbonyl-γ-L-glutamyl-L-glutamic acid. The initial dipeptide was treated with ethyl chloroformate in the presence of triethylamine. After the hydrogenolysis of the benzyloxycarbonyl group over a palladium catalyst, the catalyst was filtered off and the solution of the mixed anhydride of the dipeptide was divided into two parts. The first part was allowed to stand for 24 h at room temperature, after which the solvent was distilled off in a vacuum and the residue was further heated for 1 h also in a vacuum. The

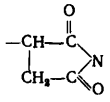
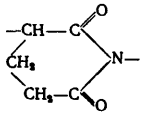
TABLE 9. Properties of *N*-phenylthiocarbonylamino-acids and their esters ($C_6H_5S-\overset{\overset{O}{\|}}{C}-HN-\underset{\underset{R}{|}}{CH}-COOH$ and $C_6H_5S-\overset{\overset{O}{\|}}{C}-HN-\underset{\underset{R}{|}}{CH}-COOR'$).

N-Phenylthiocarbonylamino-acids			Esters of *N*-phenylthiocarbonylamino-acids		
initial amino-acid	R	properties	R′	properties	references
β-Alanine	H	m.p. 104°	C_2H_5	m.p. 52°	101
DL-Phenylalanine	$C_6H_5CH_2$	m.p. 56°	C_2H_5	m.p. 63–65°	101
L-Phenylalanine	$C_6H_5CH_2$	m.p. 66°, $[\alpha]_D^{17}$ 12.5°	C_2H_5	m.p. 55–56°, $[\alpha]_D^{18}$ 14.3° (C_2H_5OH)	121
DL-Valine	$(CH_3)_2CH$	m.p. 121°	C_2H_5	m.p. 54°	121
L-Valine	$(CH_3)_2CH$	m.p. 115°, $[\alpha]_D^{17}$ 6.0° (C_2H_5OH)	C_2H_5	m.p. 60°, $[\alpha]_D^{18}$ 42.9° (C_2H_5OH)	121
L-Leucine	$(CH_3)_2CHCH_2$		C_2H_5	oil	101
DL-Methionine	$CH_3SCH_2CH_2$	m.p. 99°	C_2H_5	oil	121
L-Methionine	$CH_3SCH_2CH_2$	m.p. 65°, $[\alpha]_D^{18}$ 8.9° (C_2H_5OH)	C_2H_5	m.p. 53°, $[\alpha]_D^{18}$ 38.1° (C_2H_5OH)	121
O-Benzyl-DL-serine	$C_6H_5CH_2OCH_2$	–	CH_3	m.p. 73–74°	116
ξ-*N*-Benzyloxycarbonyl-DL-lysine	$C_6H_5CH_2OCOHN(CH_2)_4$	m.p. 116–118°	C_2H_5	m.p. 174–175°	110
L-Nitroarginine	–	m.p. 134–136°	C_2H_5	m.p. 59–60°, $[\alpha]_D^{15}$ 9.4°	118
L-Aspartic acid	$HOOCCH_2$	m.p. 100–102°		–	119
γ-Aminobutyric acid	–	m.p. 110–111°	C_2H_5	oil	113
δ-Aminovaleric acid	–	m.p. 125°	C_2H_5		114
ξ-Aminohexanoic acid	–	m.p. 95°			111
Ditto	–	m.p. 97–98°	C_2H_5	oil	109
DL-Alanylglycine	CH_3	m.p. 136–137°	C_2H_5	m.p. 119–120°	121
Glycyl-DL-alanine	CH_3	m.p. 156°	CH_3	m.p. 122–123°	107
Ditto		–	C_2H_5	m.p. 109–110°	107
L-Leucyl-L-alanine	$(CH_3)_2CHCH_2$ and CH_3	m.p. 147–148° $[\alpha]_D^{27}$ 30.8° (C_2H_5OH 1.95 g/100 ml)		m.p. 105–106° $[\alpha]_D^{27}$ 39.8° (C_2H_5OH 2.23 g/100 ml)	111
Glycyl-DL-phenylalanine	$C_6H_5CH_2$	m.p. 178–179°	C_2H_5	m.p. 107–108°	107
Glycyl-L-leucine	$(CH_3)_2CHCH_2$	m.p. 155°, $[\alpha]_D^{26}$ 15.0° (C_2H_5OH)	C_2H_5	oil	107
β-Alanyl-DL-alanine	CH_3	m.p. 146.5°	CH_3	m.p. 112–113°	108
γ-Aminobutyryl-DL-alanine	CH_3	m.p. 113°	CH_3	m.p. 95°	113
ε-Aminohexanoyl-L-phenylalanine	$C_6H_5CH_2$	m.p. 130–131°, $[\alpha]_D^{27}$ 24.1° (C_2H_5OH)	C_2H_5	oil	109
ε-Aminohexanoyl-DL-alanine	CH_3	m.p. 133–134°	CH_3	m.p. 80–82°	110
γ-Aminobutyryl-γ-aminobutyric acid		m.p. 146°	C_2H_5	m.p. 127°	113
δ-Aminovaleryl-δ-aminovaleric acid		m.p. 152–153°	C_2H_5	m.p. 97–98°	114
δ-Aminovaleryl-DL-alanine	CH_3	m.p. 155°	CH_3	m.p. 115°	114
ε-Aminohexanoylglycine		m.p. 151°	C_2H_5	m.p. 105°	115
ε-Aminohexanoyl-L-leucine	$(CH_3)_2CHCH_2$	oil, $[\alpha]_D^{16}$ 20.4° (CH_3COOH)	C_2H_5	oil	115
ε-Aminohexanoyl-α-aminobutyric acid	C_2H_5	m.p. 137°	C_2H_5	m.p. 89°	115

TABLE 10. Properties of polypeptides prepared by the thermal polycondensation of *N*-phenylthiocarbonylamino-acids [$C_6H_5SCOHNCH(R)COOH$].

Initial amino-acid	Formula of the structural unit of polypeptide	Conditions of preparation of polypeptide	Properties of polypeptide	References
1	2	3	4	5
Glycine	$-HNCH_2CO-$	in benzene with pyridine, 150 h at 80°	M_W* = 135 200, yield 91%	104
DL-Alanine	$-HNCH(CH_3)CO-$	in benzene with pyridine, 1000 h at 60°	M_W = 22 400, yield 69.6%	105
β-Alanine	$-HNCH_2CH_2CO-$	in benzene with pyridine, 1000 h at 60°	M_W = 37 300, yield 38.2%	108
»	$-HNCH_2CH_2CO-$	20 h in the melt at 120° and 3 h at 165° in a vacuum	M_W = 43 500, yield 93.5%, readily adsorbs $FeCl_3$ in HCOOH	108
DL-Phenylalanine	$-HNCH(CH_2C_6H_5)CO-$	in benzene with pyridine, 200 h at 80°	M_W = 59 400, yield 38%	103
L-Phenylalanine		in dioxan, 1000 h at 60–70°	M_W = 16 800, $[\alpha]_D^{18}$ 24.4°($ClCH_2COOH$)	121
DL-Valine	$-HNCH(CH(CH_3)_2)CO-$	ditto	M_W = 17 600	121
L-Valine	$-HNCH(CH(CH_3)_2)CO-$	»	M_W = 12 300, $[\alpha]_D^{18}$ 29.3°($ClCH_2COOH$)	121

TABLE 10 (contd.)

Initial amino-acid	Formula of the structural unit of polypeptide	Conditions of preparation of polypeptide	Properties of polypeptide	References
1	2	3	4	5
L-Leucine	—HNCHCO— $CH_2CH(CH_3)_2$	in benzene, 670 h at 80°	M_W = 26 889, yield 63%	106
L-Aspartic		in the melt at 140°, 30 h in a vacuum	M_W = 1500, yield 71%	119
L-Glutamic acid		in the melt at 180°, 20 h in a vacuum	M_W = 2100	120
DL-Methionine	—HNCHCO— $CH_2CH_2SCH_3$	in dioxan, 1000 h at 60–70°	M_W = 10500, yield 38.7%	121
L-Methionine	—HNCHCO— $CH_2CH_2SCH_3$	ditto	M_W = 23 700, $[\alpha]_D^{19}$ + 2.6° ($ClCH_2COOH$)	121
ξ-Aminohexanoic acid	—HN$(CH_2)_5$ CO—	23 h in the melt at 130° followed by vacuum treatment at 160°	M_W = 26 000, yield 77%, $[\eta]$ = 0.194 in 1 : 1 CH_3COOH + $ClCH_2COOH$	109
O-Benzylserine	—HNCHCO— $CH_2OCH_2C_6H_5$	in benzene with pyridine, 150 h at 70°	M_W = 4900–5300, m.p. 185–190°, readily debenzylated by HBr in dioxan to poly-DL-serine	116
ε-*N*-Benzyloxycarbonyl--DL-lysine	—HNCHCO— $(CH_2)_4$ $NHCOOCH_2C_6H_5$	in benzene with pyridine, 400 h at 70°	M_W = 4000, yield 48%	117
L-Nitroarginine	—HN—CHCO— $(CH_2)_3NHC(=N)NH$	in dioxan with pyridine, 16 days at 70°	M_W = 1040, $[\alpha]_D^{13}$ – 14.1°	118
Glycine + L-leucine	—HN—CH_2COHNCH—CO— $CH_2CH(CH_3)_2$	in dioxan with pyridine, 1000 h at 60°	~1 : 1 copolymer, M_W = 11 457, yield 36%	106
L-Leucine + + DL-phenylalanine	—HNCHCOHNCHCO— $CH_2CH(CH_3)_2CH_2C_6H_5$	in benzene, 1000 h at 60°	copolymer, M_W = 16 000, yield 47.8%	106
L-Leucyl-L-alanine	$CH_2C_6H_5$ —HNCHCOHNCHCO $CH_2CH(CH_3)_2$	in dioxan, 1500 h at 60–70°	M_W = 14 300, yield 24.6%	111
Glycyl-DL-alanine	—HNCH_2COHNCH(CH_3)CO— CH_3	in dioxan with pyridine, 1000 h at 60°	M_W = 34 813	107
Glycyl-DL-phenyl-alanine	—HNCH_2COHNCHCO— $CH_2C_6H_5$	ditto	M_W = 30 603	107
Glycyl-L-leucine	—HNCH_2COHNCHCO— $CH_2CH(CH_3)_2$	»	M_W = 38 000	107
β-Alanyl-DL-alanine	—HNCH_2CH_2COHNCH(CH_3)CO—	27 h in dioxan at 135° and 2 h at 150° in a vacuum	M_W = 26 100, yield 52%	108
ε-Aminohexanoyl-L--phenylalanine	—HN$(CH_2)_5$COHNCHCO— $CH_2C_6H_5$	in dioxan, 30 h at 130–140°	copolymer, M_W = 51 200, yield 79%	109
ε-Aminohexanoyl-DL--alanine	—HN$(CH_2)_5$CONHCHCH_3CO—	27 h in dioxan at 135° and 2 h at 150° in a vacuum	copolymer, M_W = 65 000, yield 82.8%; the copolymer undergoes reversible thermal denaturation, which is a maximum at 50°, heat of denaturation 45 kcal mole^{-1}	110, 112
γ-Aminobutyric acid	—NH$(CH_2)_3$CO—	in dioxan, 1.7 h at 70°	M_W = 15 000 (CH_3COOH), m.p. 230°, yield 28.6%	113
γ-Aminobutyryl-γ--aminobutyric acid	—HN$(CH_2)_3$COHN$(CH_2)_3$CO—	in dioxan, 100 h at 140°	M_W = 16 500	113
γ-Aminobutyryl-DL--alanine	—HN$(CH_2)_3$COHNCHCH_3CO—	ditto	copolymer, M_W = 71 500	113
δ-Aminovaleryl-δ--aminovaleric acid	—HN$(CH_2)_4$COHN$(CH_2)_4$CO—	»	M_W = 13 100, yield 69.5%	114
δ-Aminovaleryl-DL--alanine	—HN$(CH_2)_4$COHNCHCH_3CO—	»	copolymer, M_W = 26 000	114
ε-Aminohexanoyl-glycine	—HN$(CH_2)_5$COHNCH_2CO—	in dioxan, 100 h at 140° in an atmosphere of CO_2	copolymer, M_W = 19 300	115
ε-Aminohexanoyl-leucine	—HN$(CH_2)_5$COHNCHCO— $CH_2CH(CH_3)_2$	ditto	copolymer, M_W = 22 300, $[\eta]$ = 0.257 (30° in CH_3COOH), $[\alpha]_D^{15}$ = 32.7° (CH_3COOH)	115
ε-Aminohexanoyl-α--aminobutyric acid	—HN$(CH_2)_5$COHNCH(C_2H_5)CO—	»	copolymer, M_W = 34 900, $[\eta]$ = 0.3 (30° in CH_3COOH), undergoes reversible thermal denaturation in aqueous solutions	115

* M_W is the weight-average molecular weight.

TABLE 11. Polycondensation of $XC_6H_4OCOHNCH(CH_3).COOH$.

X	Medium	Temperature, °C	Time, h	Yield, %	Intrinsic viscosity*
H	pyridine–benzene	80	10 000	83.4	0.134
H	pyridine–toluene	100	50	92.8	0.150
H	nitrobenzene	100	16	87.1	0.286
H	»	120	4.5	96.8	0.274
H	»	125	8	73.6	0.367
o-CH_3	»	110	30	79.0	0.280
p-CH_3	»	110	56.5	82.0	0.295
o-NO_2	pyridine–toluene	100	8.0	85.3	0.113
o-NO_2	nitrobenzene	100	12.0	62.7	0.119
p-NO_2	pyridine–toluene	100	36 0	57.4	0.155
p-NO_2	nitrobenzene	100	12.0	38.4	0.203

* The viscosity of 0.2% solution in 80% dichloroacetic acid was determined.

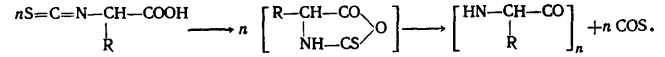
second half of the solution was treated similarly after the addition of triethylamine. In order to isolate the free polymeric acid, the polypeptides were treated in the cold with alkali and purified via the copper salt with subsequent dialysis. The molecular weight of the polypeptide obtained without triethylamine was 12 180 and that obtained with triethylamine was 20 000.

The polypeptides very tenaciously retain water and after drying in a vacuum over phosphorus pentoxide at room temperature contain one molecule of water per glutamic acid residue. Both products were colourless powders, readily soluble in water and gave positive biuret reactions and a weak ninhydrin reaction.

The amino-group can also be activated by treating amino--acids with isocyanates. The resulting amide of the alkanecarbamic acid, with the general formula

RNHCONHCRHCOOH,

where R = t-alkyl, breaks down at a temperature above 175° with the formation of a polypeptide in more than 80% yield. The degree of polycondensation of polypeptides prepared in this way reaches 100.[131] An interesting synthesis of polypeptides is by the polycondensation of the isothiocyanates of aliphatic carboxylic acids, prepared by treating α-amino-acids with carbon disulphide and then decomposing the dithiocarbamic derivatives with mercuric chloride[132]. However, in this case it is assumed that heating of the isothiocyanate leads to the formation of the anhydride of the *N*-thiocarboxy-acid and its polymerisation and not the removal of the substituent from the nitrogen atom:



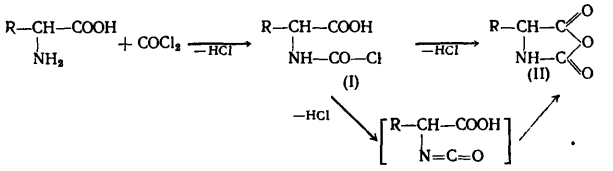
IV. SYNTHESIS OF POLYPEPTIDES BY THE POLYMERISATION OF THE ANHYDRIDES OF *N*-CARBOXY--α-AMINO-ACIDS

1. Synthesis of the Anhydrides of *N*-Carboxy-α-amino--Acids

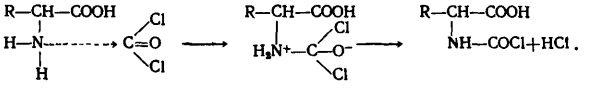
The most usual synthesis of polypeptides is by the polymerisation of the anhydrides of *N*-carboxy-α-amino-acids, which permit the preparation of polymers with a specified molecular weight, capable of being varied over a very wide range, with complete elimination of the racemisation of the amino-acids.

The most widely used procedure for the preparation of the cyclic anhydrides of *N*-carboxy-α-amino-acids is that proposed by Fuchs[133], Farthing[134], and Levy[135]. Finely ground amino-acids, their chlorides, or sodium salts, suspended in an inert solvent (dioxan, ethyl acetate) are treated with phosgene at 30–50°. The reaction may be carried out with simultaneous addition of equivalent amounts of a solution of phosgene in dioxan and a suspension of the amino-acid in dioxan to an excess of the solvent[136]. The anhydrides of the *N*-carboxy-acids and their thio-analogues are also obtained on heating the chlorides of amino-acids with $COCl_2$ or $CSCl_2$ in nitrobenzene[137].

The reaction of α-amino-acids with phosgene proceeds as follows:



The first stage, the formation of the carbamoyl chloride (I) begins with the nucleophilic attack by the amino-nitrogen on the carbonyl carbon in phosgene:

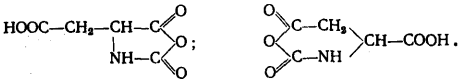


The rate of this process should increase with increase in the electron-donor effect of the R group in the amino-acid, which enhances the basicity of the amino-group. This hypothesis agrees satisfactorily with the observed increase in the rate of reaction with phosgene in the series glycine < alanine < < α-aminobutyric acid ≃ phenylalanine < norleucine < < leucine < isoleucine < pseudoleucine (*C*-t-butylglycine), where the substituent R varies from H to $(CH_3)_3C-$.[138,139]

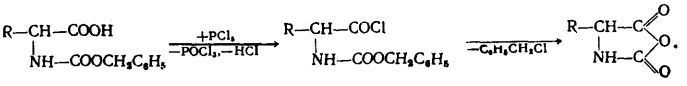
Preliminary saturation of dioxan with sulphur dioxide[140] and also the addition of small amounts of acetic anhydride increase the rate of reaction with phosgene, while impurities may retard it to a large extent[138].

The stage of the cyclisation of the carbamoyl chloride (I) to the anhydride (II) of the *N*-carboxy-α-amino-acid occurs spontaneously and only in the case of proline and its derivatives, where the closure of the second ring involves considerable stresses in the molecule, is additional treatment of the carbamoyl chloride with triethylamine[141] or silver oxide[142,143] necessary.

In the synthesis of the anhydrides of trifunctional *N*-carboxy-α-amino-acids it is necessary to protect the functional groups capable of reacting with phosgene[144]. In the absence of protection aspartic acid gives a mixture of five- and six-membered anhydrides of the *N*-carboxy--acid[145]:



The anhydrides of *N*-carboxy-α-amino-acid are also obtained by treatment of *N*-alkoxycarbonyl- and particularly *N*-benzyloxycarbonyl-α-amino-acids with thionyl chloride, phosphorus pentachloride, or phosphorus tribromide:

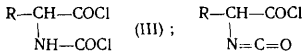
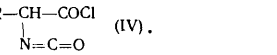


This method[3] is usually employed for β-amino-acids, the *N*-carboxy-derivatives of which yield six-membered anhydrides capable of polymerising[148].

An interesting method of preparation of the anhydrides of the *N*-carboxy-derivatives of various amino-acids[149,150] involves the reaction of thionyl chloride or phosgene with the disodium salt of the *N*-carboxy-amino-acid which is readily obtained from sodium carbonate and the amino-acid or by the simultaneous treatment of the amino-acid with sodium methoxide and carbon dioxide[134,147,148].

The synthesis of the anhydrides of *N*-carboxy-α-amino--acids from the malonic ester[151,152] is suitable only for the preparation of racemic products and has not been used in recent years.

High-molecular-weight polypeptides can be synthesised only from thoroughly purified anhydrides of *N*-carboxy-α--amino-acids which in their synthesis by the reaction of phosgene with α-amino-acids are usually contaminated with the dichloride (III) of the *N*-carboxy-α-amino-acid and the isocyanate (IV) formed from it:

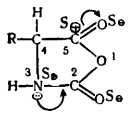

(III);

(IV).

However, the impurities are readily removed on recrystallisation[153] or sublimation in a high vacuum. The removal of chlorine-containing impurities by treating a solution of the anhydride of the *N*-carboxy-α-amino-acid in ethyl acetate with the copolymer of ethyl β-(formyl-*N*-methylamino)-acrylate and divinylbenzene has been described[154]. The anhydrides of *N*-carboxy-α-amino-acids are kept at a temperature not exceeding -30°. The anhydrides of *N*-carboxy--α-amino-acids whose synthesis has been described in recent years are listed in Table 12.

The anhydrides of *N*-carboxy-α-amino-acids are determined quantitatively by titration in a non-aqueous medium with sodium methoxide[155,156] or by the determination of the carbon dioxide evolved on their acid hydrolysis[157,158]; the carbon dioxide is absorbed by an ethanol–dioxan solution of benzylamine to form the benzylammonium salt of toluene--ω-carbamic acid, and then titrated with a solution of sodium methoxide using Thymol Blue as the indicator.

2. Chemical Properties of the Anhydrides of *N*-Carboxy--α-amino-Acids

The anhydrides of *N*-carboxy-α-amino-acids are extremely reactive. They have the structure of 2,5-dioxo-oxazolidine substituted in position 4.



The electronegativity of the oxygen atom gives rise to partial positive charges on carbon atom 5 and the nitrogen atom which results in a weakening of the N–H bond and great enhancement of the reactivity of the carbonyl carbon atom 5 towards nucleophilic reagents compared with carbon atom 2. Indeed, nucleophilic addition to the carbonyl carbon atom 5 and reactions involving the participation of the N–H group are the most characteristic reactions of the anhydrides of *N*-carboxy-α-amino-acids.

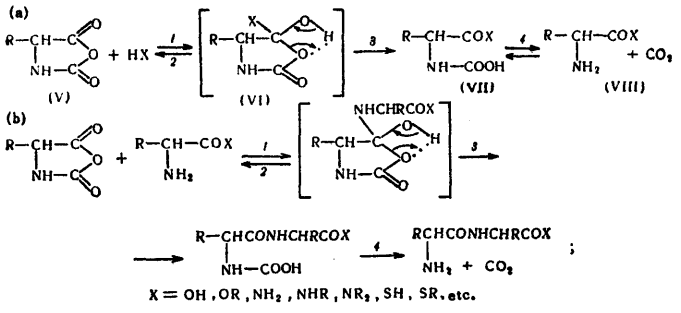
TABLE 12. Synthesis of the anhydrides of *N*-carboxy-α-amino--acids not included in the monograph of Bamford et al.[4] and the review of Katchalski and Sela[5].

Initial α-amino-acid	Method of preparation	Method of purification	M.p., °C	References
1	2	3	4	5
Glycine	phosgene treatment			138,140
»	Na salt of *N*-carboxy-glycine + $SOCl_2$	sublimation		150
Sarcosine	Na salt of *N*-carboxy-sarcosine + $SOCl_2$	»		150
DL-*C*-Phenylglycine	Na salt of *N*-carboxy-phenylglycine + + $SOCl_2$	»		150
DL-*C*-(*p*-Nitrophenyl)-glycine	phosgene treatment	ethyl acetate	135	150
DL-*C*-Propargylglycine	ditto	tetrahydrofuran–petroleum ether	125–127	299
DL-Alanine	»		42–44	138,140
Dehydroalanine	*N*-benzyloxycarbonyl-dehydroalanine + PCl_5	ethyl acetate–petroleum ether, sublimation	111–113	303
DL-Phenylalanine	phosgene treatment		126	138,279
»	Na salt of *N*-carboxy-phenylalanine + + $SOCl_2$	sublimation		150
»	*N*-benzyloxycarbonyl-phenylalanine + PCl_5	ether	123–125 (decomp.)	173
DL-α-Aminobutyric acid	phosgene treatment		112–113	138
DL-Valine	ditto			140
»	*N*-benzyloxycarbonyl-valine + PCl_5	ether	80–81 (decomp.)	173
DL-Norvaline	*N*-benzyloxycarbonyl-norvaline + PCl_5	ether	67–69 (decomp.)	173
L-Leucine	phosgene treatment			138
DL-Leucine	*N*-benxyloxycarbonyl-leucine + PCl_5	ether	48–50	173
DL-Norleucine	*N*-benzyloxycarbonyl-norleucine + PCl_5	ether	78–80	173
»	phosgene treatment		79	138
DL-Isoleucine	ditto		70–71	138
DL-Pseudoleucine	»		120	138
L-Proline	phosgene treatment, with $(C_2H_5)_3N$	ethyl acetate or sublimation	45	141
DL-Serine	phosgene treatment	ethyl acetate–hexane	82–83	286
»	ditto	benzene	115 (decomp.)	286
O-Acetyl-DL-serine	»	ethyl acetate–hexane	62.5–63.5 (decomp.)	286
O-Acetyl-L-serine	»	ditto	50–51	286
O-Benzyl-L-serine	»	ether–petroleum ether	72 (decomp.)	301
O-Benzyl-L-tyrosine	»		142 (decomp.)	308
O-Benzyloxycarbonyl--L-tyrosine	»			140
O-Phenyl-DL--homoserine	»	ethyl acetate–petroleum ether	105–106	310
»	*N*-benzyloxycarbonyl--*O*-phenylhomoserine + + PCl_5	ditto	105	310
ε-*N*-Benzyloxycarbonyl--L-lysine	phosgene treatment	ethyl acetate–hexane	101–101.5	281
»	ditto		98.5–99.5	140
ε-*N*-Benzyloxycarbonyl--DL-lysine	»		100	305
δ-*N*-Benzyloxycarbonyl--L-ornithine	»		86	307
β-Benzyl L-aspartate	»	dichloroethane	126	297
Ditto	»	ethyl acetate–hexane	127–128	144
»	β-benzyl *N*-benzyl-oxycarbonylaspartate + + PCl_5	ditto	127–128	144
β-Benzyl DL-aspartate	phosgene treatment of dibenzyl ester	»	126–127.5	144
β-*p*-Nitrobenzyl L-aspartate	phosgene treatment			302
β-Peptido-DL-aspartic acid	ditto			300
γ-Ethyl L-glutamate	γ-ethyl *N*-benzyloxy-carbonylglutamate + + $SOCl_2$	ethyl acetate		182

TABLE 12 (contd.)

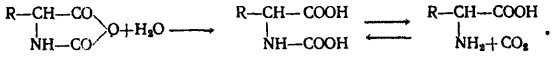
Initial α-amino-acid	Method of preparation	Method of purification	M.p., °C	References
1	2	3	4	5
δ-Methyl DL-α-amino-adipate	phosgene treatment	chloroform–petroleum ether	94–95	309
DL-Methionine	ditto			138
S-Methyl-L-cysteine	»	ethyl acetate–petroleum ether	74	311
S-Ethyl-L-cysteine	»		69	311
S-Propyl-L-cysteine	»			311
S-Isopropyl-L-cysteine	»			311
S-Butyl-L-cysteine	»	ethyl acetate–petroleum ether	55–56	311
S-Pentyl-L-cysteine	»			311
S-Hexyl-L-cysteine	»	ether–petroleum ether	42–43	311
S-(α-Methylnaphthyl)-L-cysteine	»	ethyl acetate–petroleum ether	105	311
S-Dodecyl-L-cysteine	»	ditto	66–67	311
S-Benzyl-L-cysteine	»			298
S-Phenyl-L-cysteine	»			298
S-*p*-Tolyl-L-cysteine	»			298
S-Phenylthio-L-cysteine	»	ethyl acetate–petroleum ether	117–119	304
γ-Benzyl ester of *S*-carboxy-L-cysteine	»			306
δ-Benzyl ester of *S*-methoxycarbonyl-L-cysteine	»		109	306
ε-Benzyl ester of *S*-ethoxycarbonyl-L-cysteine	»			306
Mono-*N*-Benzyloxycarbonyl-L-cysteine	»	ether–petroleum ether	30–32	312
Monoethyl ester of *N*-acetyllanthionine	»			312
Benzyl ester of *N*-benzyloxycarbonyllanthionine	»			312
S-Benzyloxycarbonyl-DL-homocysteine	»	ether–petroleum ether	75–76	313
S-Benzyl-DL-homocysteine	»	ditto	95–96	313
S-Benzylhomo-DL-cysteine	»	»	57	313
S-Ethyl-DL-homocysteine	»			313
S-Propyl-DL-homocysteine	»			313
S-Butyl-DL-homocysteine	»			313
S-Allyl-DL-homocysteine	»			313

The reaction with nucleophilic reagents HX usually proceeds as follows:



Compound (VIII) has a free amino-group and may react with another molecule of the anhydride like any nucleophilic reagent. Therefore in the presence of a large excess of the anhydride of the *N*-carboxy-α-amino-acid this reaction results in the formation of a polypeptide. In the presence of an excess of HX only compounds of the type of (VIII) are formed.

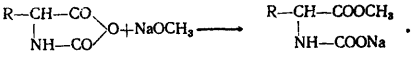
In the reaction with water, in which many anhydrides of *N*-carboxy-α-amino-acids are quite soluble, the following equilibrium is established at low temperatures [3,159-162]:



It has been shown that the decomposition of the anhydrides of *N*-carboxy-α-amino-acid is catalysed by bases and acids [163].

Hydrogen sulphide, alcohols, phenols, and mercaptans react in the presence of triethylamine [164,165] similarly to water with the formation of α-amino-thioacids, esters, and thioesters.

Anhydrous hydrogen chloride in an inert medium reacts with the anhydrides of *N*-carboxy-α-amino-acids to yield the hydrochlorides of the amino-acid chlorides [166,167] while in alcoholic medium it accelerates alcoholysis, a fact which has been used for the preparation of the esters of various α-amino-acids [168-172]. Alcoholysis is also accelerated by alkoxides, a finding on which the titration of the anhydrides of *N*-carboxy-α-amino-acids with sodium methoxide is based [155]:



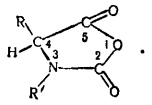
Since primary and secondary amines are nucleophilic agents, they react with the anhydrides of *N*-carboxy-α-amino-acids according to mechanism (a) given above with the formation of peptides and amino-acid amides [133,173-177]. The thio-analogues of the anhydrides of *N*-carboxy-α-amino-acids are capable of the formation of a number of other products [178].

The rate of reaction with nucleophilic reagents decreases with decreasing basicity in the following order: CH_3O^-, OH^-, RNH_2, $ArNH_2$, ROH, H_2O. In the reaction with the last three members of the series the free amino-group formed after their addition is more nucleophilic and readily reacts with the free molecules of the anhydrides of *N*-carboxy-α-amino-acids according to mechanism (b) with the formation of oligomers and polymers even in the presence of an excess of the above reagents [175].

The rate of reaction depends also on the nature of the *N*-carboxy-α-amino-acid anhydride and is greater the greater the partial positive charge on the carbonyl carbon atom 5 and the lower the steric hindrance for the attacking nucleophilic agent. For this reason, the reactivity and the rate of polymerisation of the anhydride diminish with increase in the electron-donor effect and particularly with increase in the size of the substituent R, in the following series: glycine, alanine, α-aminobutyric acid, phenylalanine, norleucine, leucine, isoleucine, pseudoleucine, and α-methylalanine [138,139,179].

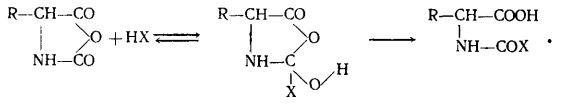
The considerable influence of steric factors on the rate of reaction of the anhydrides of *N*-carboxy-α-amino-acids with primary and secondary amines has been demonstrated by Ballard and Bamford [179]. The usual five-membered ring of the *N*-carboxy-α-amino-acid anhydride, which incorporates nitrogen, oxygen, and two trigonal carbon atoms, is planar and unstrained, the substituent R lying to one side or another of the molecule depending on the asymmetry of carbon atom 4. In the case of *N*- substituted

anhydrides the substituent R′ at the nitrogen is located in the *trans*-position relative to R.



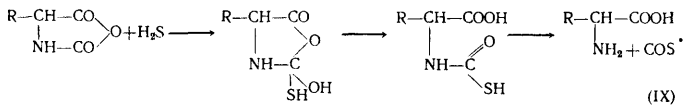
The attacking reagent approaches the carbon atom 5 from the side opposite to that of the carbonyl oxygen along the line passing at a small angle to the plane of the ring in the immediate vicinity of the nitrogen atom. The presence of the substituents R and R′ gives rise to considerable steric hindrance to attack from both sides of the ring, particularly in the case of bulky nucleophilic agents such as secondary amines, and sharply reduces the rate of reaction. In the anhydrides of *N*-carboxyproline and its derivatives both substituents lie on one side of the plane of the ring, which is responsible for the enhanced rate of their reaction with secondary amines.

Similarly, the second carbonyl group can also react with nucleophilic agents: with the formation of urethanes, thiourethanes, and urea derivatives in the case of alcohols, mercaptans, and amines respectively.



However, by virtue of the smaller positive charge of carbon atom 2 the activation energy for this reaction is much higher and it becomes important only at high temperatures and concentration, and when the reactivity of the nucleophilic reagent is high; steric factors can also have an appreciable effect.

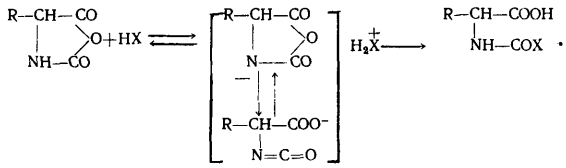
The addition to the carbonyl carbon atom 2 is usually referred to as "erroneous", leading to the disappearance of the amino-group at the end of the growing chain and termination of the polymerisation. The possibility of this reaction was confirmed by Berger et al.[155], who observed the formation of urethane in the reaction of the anhydride of *N*-carboxyphenylalanine with a solution of sodium methoxide. Wieland and Euler[165] allowed the anhydrides of *N*-carboxy-α-amino-acids to react with dry hydrogen sulphide in the presence of triethylamine and obtained α-aminothio-acids, which were always contaminated with α-amino-acids, whose formation can be explained by the decomposition of the corresponding thiocarbamic acids (IX):



(IX)

In the presence of a large excess of amines the addition to carbon atom 2 can occur to a considerable extent[180,181] but the amount of the product of the normal addition rapidly increases when the concentration and basicity of the amine are reduced[180].

However, the anhydride of *N*-carboxysarcosine combines even with nucleophilic agents as powerful as methoxide ions only at carbonyl carbon atom 5.[181] Possibly, the formation of compounds of the type RCH(COOH)NHCOX requires a hydrogen atom linked to the nitrogen atom. In this case nucleophilic attack can occur not only at the carbonyl carbon atom 2 but also at the hydrogen atom, with formation of carbamic acid derivatives via the isocyanate stage[165,181]:



The increase of the affinity of the base HX for a proton should favour this reaction, which agrees with experimental findings[181,182].

The active role of the NH group is particularly evident in the reaction of the anhydrides of *N*-carboxy-α-amino--acids with Grignard reagents[183]. In the *N*-substituted *N*-carboxy-α-amino-acid anhydrides the carbonyl group in position 2 does not react with RMgX and the anhydride of *N*-carboxy-*N*-phenylglycine gives only the following compounds:

$$C_6H_5NHCH_2—COOR,\quad C_6H_5NHCH_2—C(OH)R_2\ (R=CH_3,\ C_2H_5,\ C_6H_5).$$

At the same time the anhydride of *N*-carboxyphenylalanine forms under the same conditions *N*-acylated amino-ketones and amino-alcohols:

$$C_6H_5CH_2CH(COR)NHCOR\quad C_6H_5CH_2CH(NHCOR)C(OH)R_2.\ (R=C_2H_5,\ C_6H_5)$$

Among other reactions of the anhydrides of *N*-carboxy--α-amino-acids unrelated to their polymerisation, their reduction with lithium aluminium hydride[184] and their application in the Friedel–Crafts synthesis have been described[185,186].

3. Polymerisation of the Anhydrides of *N*-Carboxy-α--amino-Acids†

The anhydrides of *N*-carboxy-α-amino-acids readily polymerise both in the solid and in solution. The molecular weight and the polydispersity of the polypeptides formed are determined by the nature of the initiator and the polymerisation conditions. Solid polymerisation is usually carried out at elevated temperatures and low-molecular--weight products are obtained. Inert solvents are used for polymerisation in solution, which is carried out at low temperatures, the initiators being amines, alkoxides, alkalis, or water. The initiators and inhibitors of radical type polymerisation do not have any influence on the polymerisation of the anhydrides of *N*-carboxy-α-amino-acids[187,188]. Primary and secondary amines yield polymers with the degree of polymerisation up to 200, depending on the amount of amine.

Using excess amounts of amino-acid esters in the reaction with the anhydrides of *N*-carboxy-α-amino-acids, it is possible to obtain dipeptides[175,189,190]. However, in this case it is difficult to avoid the formation of higher

† It should be noted that the polymerisation of the anhydrides of *N*-carboxy-α-amino-acids differs considerably from the classical example of polymerisation and polycondensation. A better name for it would be polyaddition with subsequent removal of some of the molecules.

oligopeptides [191] and urea derivatives [192]. At low temperatures (-40°) in the presence of triethylamine, which stabilises by salt formation the carbamic acid (VII) formed in the first addition reaction, it proved possible to synthesise di- and tri-peptides in good yields [193-198]. The use of the thio-analogues of the anhydrides of *N*-carboxy-α-amino-acids is also promising for stepwise polypeptide synthesis of this kind [178].

Polypeptides of the highest molecular weight are obtained in the polymerisation of the anhydrides of *N*-carboxy-α-amino-acids in the presence of tertiary amines, alkoxides, and alkali metal hydroxides. However, the reproducibility of the results and the molecular weight of the polypeptides depend also on the purity of the initial compounds and solvents. Impurities, for example chloride ions at concentrations of about 0.01%, can completely vitiate the results of the experiment [199].

Because it is impossible to determine the impurities at such concentrations by the usual methods, it is recommended that the purity of the anhydrides of *N*-carboxy-α-amino-acids be checked by their polymerisation under standard conditions. Thus Idelson and Blout [200] regarded the anhydride of γ-benzyl *N*-carboxy-L-glutamate to be pure if at room temperature in 4% dioxan solution it gave a polymer with a molecular weight not less than 360 000, using sodium methoxide as the initiator with $[I_0]/[M_0] = 1:200$ (see below).

The polymerisation of the anhydrides of *N*-carboxy-α-amino-acids is inhibited by a large number of compounds: boric and dichloroacetic acids [201], hydrogen chloride [202], aldehydes [203,204], phenyl isocyanate, acetic anhydride, etc. Acids inhibit the reaction [205], even if it is initiated by inorganic salts [206,207]. Acetyl chloride, thionyl chloride, aluminium chloride, etc. fully inhibit the polymerisation of the anhydride of *N*-carboxyglycine when present in concentrations up to 0.2 mole %. [208]

The polypeptides obtained under different conditions by the polymerisation of the anhydrides of *N*-carboxy-α-amino-acids, and some of their properties, are listed in Table 13.

A. Polymerisation in the solid form. The anhydrides of *N*-carboxy-α-amino-acids are stable only at temperatures below -30°. At room temperature under the influence of atmospheric moisture the anhydrides of the *N*-carboxy-derivatives of glycine [3,174,210], L-alanine [211], L-leucine [209,211], *C*-phenylglycine, and phenylalanine [209] are converted into polymers. The rate of polymerisation is

TABLE 13. Preparation of polypeptides by the polymerisation of the anhydrides of the *N*-carboxy-derivatives of α-amino-acids (examples not included in the monograph of Bamford et al. [4] and the review of Katchalski and Sela [5]).

Anhydride of *N*-carboxy-derivative of	Solvent	Initiator	Mol. weight of polypeptide	Removal of protecting groups	References
1	2	3	4	5	6
Glycine	dioxan	*p*-bromoaniline			227
»	»	water			138
DL-*C*-Propargylglycine	nitrobenzene	*ammonia*	$n = 32$		299
DL-Alanine		dodecylamine			229
»	dioxan	water			138
»	tetrahydrofuran	methanol, menthol, *borneole*, Al, Zn, Cd, Li compounds			295
L- and D-Alanine	benzene	tri-n-butylamine			288
Dehydroalanine	toluene, pyridine	triethylamine			315
DL-Phenylalanine	dioxan	water			138
»	»	$NaOCH_3$	⩽140 000		279
»	chloroform	$NaOCH_3$	⩽120 000		279
»	chloroform, benzene	water	⩽70 000		279
DL-α-Aminobutyric acid	dioxan	»			138
DL- and D-α-aminobutyric acid	nitrobenzene	benzylamine			288
DL- and L-Leucine	benzene	tri-n-butylamine			288
DL-Norleucine, DL-isoleucine, and DL-pseudoleucine	dioxan	water			138
L-Proline	pyridine				141
»	acetonitrile	$NaOCH_3$	50 000–90 000		316
O-Acetoxy-L-proline	»			NH_3 or $NaOCH_3$	143
L-Serine	nitrobenzene	$NaOCH_3$			286
O-Acetyl-L-serine	»	triethylamine		$NaOCH_3$	286
O-Acetyl-DL-serine	»	$NaOCH_3$		$NaOCH_3$	286
O-Benzyl-L-serine	chlorobenzene	$NaOCH_3$		HBr + dioxan	301
O-Benzyl-DL-serine	dioxan	triethylamine	$n = 28–30$	ditto	314
O-Phenyl-DL-serine		100–135°, vacuum			310
O-Benzyl-L-tyrosine	dioxan	triethylamine		$HBr + CH_3COOH$	308
O-Benzyloxycarbonyl-L-tyrosine	benzene	water		$HBr + CH_3COOH$	318
ε-*N*-Benzyloxycarbonyl-L-lysine	»	»		$HBr + CH_3COOH$	318
»	dioxan	$NaOCH_3$	2 000 000	dioxan + $CHCl_3$ + + HCl + HBr	281
ε-*N*-Benzyloxycarbonyl-DL-lysine	»	triethylamine	$n = 31–41$		305

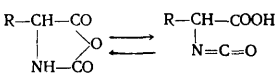
TABLE 13 (contd.)

Anhydride of *N*-carboxy-derivative of	Solvent	Initiator	Mol. weight of polypeptide	Removal of protecting groups	References
1	2	3	4	5	6
δ-*N*-Benzyloxycarbonyl-L-ornithine	dioxan		$n = 125$	HCl + CH_3COOH	307
β-Benzyl L-aspartate and D-aspartate	chloroform, methylene chloride, dichloroethane	$NaOCH_3$, benzylamine	50000		144, 296, 297
β-*p*-Nitrobenzyl-L-aspartate	dimethylformamide	diethylamine			302
β-Peptido-DL-aspartate		heating in a vacuum		LiOH + C_2H_5OH	300
γ-Methyl L-glutamate and D-glutamate	ethyl acetate	NH_4OH	13000	NaOH + $Cu(OH)_2$	317
γ-Methyl L-glutamate	chloroform	CH_3COOH	$n = 500–600$		292
γ-Benzyl L-glutamate and D-glutamate	dioxan, benzene, dimethylformamide	$NaOCH_3$, NaOH, trimethylamine	15000–100000		288, 289
δ-Methyl D-α-aminoadipate and L-α-aminoadipate	ethyl acetate	triethylamine			309
S-Methyl-L-cysteine	pyridine				311
S-Ethyl-L-cysteine	pyridine, ethanol		$n = 11–25$		311
»		130–140°, vacuum	$n = 53$		311
S-Propyl-, *S*-isopropyl-, *S*-butyl-, *S*-pentyl-, *S*-hexyl-, *S*-dodecyl-, and *S*-methylnaphthyl-L-cysteine	pyridine, dioxan, nitrobenzene, ethanol, acetic acid	heating with or without vacuum			311
S-Phenylthio-L-cysteine	ethyl thioglycolate	heating		simultaneous removal of thiophenol	304
γ-, δ-, and ε-Benzyl esters of *S*-carboxy-, *S*-methoxycarbonyl-, and *S*-ethoxycarbonyl-L-cysteine		heating in a vacuum			306
Mono-*N*-Benzyloxycarbonyl-L-cysteine		ditto		HBr + CH_3COOH	312
S-Ethyl-, *S*-propyl-, *S*-butyl-, *S*-pentyl-, *S*-benzyl-, *S*-benzoyl-, and *S*-benzyloxycarbonyl-DL-homocysteine	dioxan, pyridine	triethylamine, heating in a vacuum			313
Monoethyl ester of *N*-acetyllanthionine		heating in a vacuum			312
Monobenzyl ester of *N*-benzyloxycarbonyllanthionine	pyridine	ditto			312

determined by the structure and the temperature. The mechanism of the polymerisation of crystalline anhydrides of *N*-carboxy-α-amino-acids initiated by atmospheric moisture is as yet unknown. The shape of the crystals remains unchanged during polymerisation and only X-ray diffraction analysis can detect the alterations in the crystal lattice [212]. Wessely and Siegmund [213] isolated from the polymerisation products of the anhydride of *N*-carboxysarcosine 4% of 1,4-dimethyl-2,5-dioxopiperazine in addition to 7% of polysarcosine.

In the molten state all the anhydrides of *N*-carboxy-α-amino-acids rapidly split off carbon dioxide and are converted into polymers with a relatively low degree of polymerisation [171,214–217].

The mechanism of thermal polymerisation is obscure. Possibly the α-isocyanatocarboxylic acid, capable of polymerising with subsequent removal of carbon dioxide, is an intermediate [218].



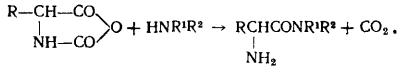
$$OCN—CHRCOOH + OCN—CHRCOOH \longrightarrow OCN—CHR—CO—O—CO—$$
$$—NHCHRCOOH \longrightarrow OCN—CHRCO—NHCHRCOOH + CO_2 .$$

The termination of the growing chain can occur as a result of the deactivation of the isocyanato-end-group.

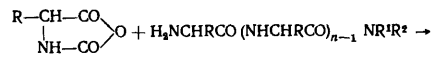
Heyns and Brockmann [219] showed that in the thermal polymerisation of [^{13}C]-*N*-carboxyglycine anhydride the carbon dioxide split off contains exclusively the second carbon atom of the ring. However, this is insufficient to settle the problem whether the polymer forms as a result of normal chain growth at the amine end or via the intermediate isocyanatocarboxylic acid, which is probably present in equilibrium with the anhydride of the *N*-carboxy-α-amino-acid [165,181]. The detection of considerable amounts of 2,5-dioxopiperazines in the polymerisation of the anhydrides of *N*-carboxyglycine and *N*-carboxyalanine at 100–165° [220] also prevents final conclusions concerning the mechanism of the reaction.

B. Polymerisation in solution. Initiation with primary and secondary amines. All the known anhydrides of *N*-carboxy-α-amino-acids, except for the anhydride of *N*-carboxy-α-methylalanine [179,247] readily polymerise in the presence of amines in inert solvents (dioxan, benzene, nitrobenzene, chloroform, acetophenone, dimethylformamide, etc.) at relatively low temperatures. The polymerisation can be divided into three main stages.

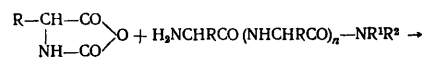
Initiation:



Chain growth:

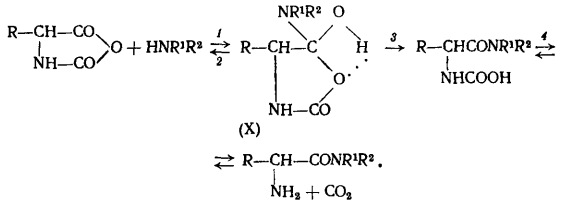


Chain termination:



The initiation and chain growth correspond to "normal" addition of nucleophilic agents to the carbonyl group in position 5, and chain termination to "erroneous" addition. The average degree of polymerisation $\overline{P}_n$ of the polypeptides formed (10–200) is usually equal to the ratio of the initial concentrations of the monomer $[M_0]$ and initiator $[I_0]$, variation of which permits the preparation of polypeptides of the required molecular weight.

Initiation proceeds according to the usual mechanism involving the addition of nucleophilic agents to the anhydrides of *N*-carboxy-α-amino-acids:



The rate of initiation depends on the rate of the individual reactions *1*, *2*, and *3*. If among the three stages reaction *3* is the slowest, equilibrium is established between the anhydride of the *N*-carboxy-α-amino-acid and compound (X). On the other hand, if the rate of reaction *1* is slow, i.e. because of its low basicity or because of steric hindrance, the initiator HNR^1R^2 adds with difficulty to the carbonyl group in position 5 and is readily split off again, stage *1* determining the rate of the overall initiation process. On the basis of measurements of the isotopic effect (see below) Heyns and Schultze [150] concluded that the anhydrides of the *N*-carboxy-derivatives of phenylalanine and sarcosine react in this way with aniline. The ratios of the rate constants of reactions *1*, *2*, and *3* are 0.08 : 75 : 15 in the case of phenylalanine and 0.07 : 86 : 10 in the case of sarcosine.

The rate of initiation may be measured in the reaction with a large excess of the amine, when the polymerisation does not have a significant effect on the rate of consumption of the anhydride. It was found that in this case the reaction is of second order.

Ballard and Bamford [221] measured the rate constants K_1 for the reactions of various anhydrides of *N*-carboxy-α--amino-acids with amines of the type $RHNCH_2CON(CH_3)_2$. The results, which are presented in Table 14, indicate a considerable effect of steric hindrance, due to substituents at the nitrogen and carbon atom 4, on the rate of reaction with secondary amines.

Direct determination of the rate of initiation in polymerisation is very difficult. It can be only approximately estimated from the overall rate of initiation and polymerisation during early stages of the process. It was thus

TABLE 14. Rate constants K_1 (litre $mole^{-1}$ s^{-1}) for the initiation of the polymerisation of the anhydrides of *N*-carboxy--α-amino-acids with amines of the type $RNHCH_2CON.(CH_3)_2$ in nitrobenzene at 15°.

Anhydride of *N*-carboxy-derivative of / R	Alanine	Sarcosine	*N*-methyl-alanine	Phenyl-alanine	*N*-isopropyl-glycine	α-Methyl-alanine	L-Proline
R= H	1.13	0.82	0.45	1.0	0.83	0.29	2.68
R= CH_3	0.48	0.57	0.16	0.40	0.48	$8.4\cdot10^{-4}$	2.00
R=$CH(CH_3)_2$	0.10	7.4	$2\cdot10^{-5}$	0.30	$<10^{-7}$	$1\cdot10^{-6}$	0.12

shown that initiation with n-hexylamine is approximately ten times faster than the polymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate in dioxan [200]. The rate constant for initiation is in this case $K_1 = 0.2 \pm 0.1$ litre × × $mole^{-1}$ s^{-1}. [199] The rate of initiation with *p*-chloroaniline of the polymerisation of the anhydride *N*-carboxy-DL-phenylalanine is smaller by a factor of 6000 than the rate of polymerisation itself, while in the case of initiation with *N*-ethylglycine diethylamide in benzene and nitrobenzene the two rates are approximately equal [222]. Relatively rapid initiation has been observed in the systems comprising the anhydride of α-*N*-carboxy-ε-*N*-benzyloxycarbonyl-L-lysine and ammonia and dioxan [223] and the anhydride of *N*-carboxy--DL-phenylalanine or *N*-carboxy-DL-leucine and the sodium salt of DL-phenylalanine [224].

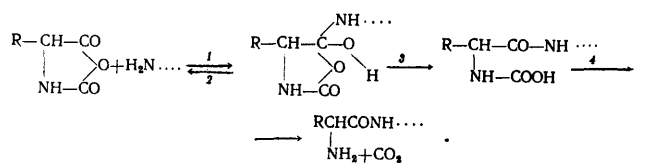
Ballard and Bamford [225] studied the effect of the peptide chain length on the rate of initiation of the polymerisation of the anhydrides of *N*-carboxy-DL-leucine and *N*-carboxy--DL-phenylalanine in nitrobenzene. They found that the dimethylamide of leucine is 3–4 times more active than the dipeptide, tripeptide, and higher peptides, the chain growth having begun proceeds at a constant rate since the reactivity of the amine of the amino-group at the chain end remains virtually constant, starting with the dimer.

It is evident from the mechanism of the initiation reaction that the initiator remains at the end of the polypeptide chain. This enabled Ritschard [226] to follow directly by means of paper chromatography the rate of consumption of the initiator in the polymerisation of γ-benzyl L-glutamate. He employed a coloured initiator — 2,4-dinitrophenylethylenediamine. From photometric measurements on such coloured polymers, their molecular weights were calculated [228]; this can also be done by determining, for example, the halogen content of polypeptides obtained in the presence of halogen-containing initiators [227].

Using as initiators polypeptides with free amino-groups or amines of large molecular weight [229], it is possible to obtain various block copolymers. The rate of initiation and chain growth are usually equal and independent of the degree of polymerisation ($\overline{P}_n$) of the initiator [225]. However, in the case of the polymerisation of the anhydride of *N*-carboxy-DL-phenylalanine initiated with polysarcosine Ballard and Bamford [230,231] observed a direct relation between the rate of polymerisation and initiator chain length up to $\overline{P}_n = 20$, polysarcosine being a hundred times more effective than the monomeric sarcosine dimethylamide. Further increase of $\overline{P}_n$ did not raise the rate of polymerisation. According to the authors, polysarcosine, which does not have intramolecular hydrogen bonds, forms hydrogen bonds with the NH groups of the anhydrides of *N*-carboxy-α-amino-acids, and this increases their concentration on the surface of polysarcosine and as a result increases the rate of polymerisation. This hypothesis has

not as yet been adequately confirmed, since the behaviour of polyproline and polyhydroxyproline, which also do not have NH groups, has not been investigated.

Chain growth. The mechanism of chain growth is similar to that of initiation:



Using radioactive carbon, it was shown that, as was to be expected, the carbon dioxide evolved during polymerisation contains carbon originating exclusively from the carbonyl in position 2 of the *N*-carboxy-α-amino-acid anhydride ring [149]. From a study of the time variation of the isotopic composition of the carbon dioxide evolved, Heyns and Schultze [150] established that the removal of carbon dioxide (stage *4*) in the polymerisation of the anhydrides of the *N*-carboxy--derivatives of glycine, sarcosine, phenylalanine, and *p*-nitrophenylglycine is relatively rapid. The slowest stage in the polymerisation of the anhydrides of the *N*-carboxy--derivatives of phenylalanine, phenylglycine, and *p*-nitrophenylglycine is reaction *1*, while in the case of glycine the stage involving ring opening (reaction *3*) is slower, and in the case of sarcosine the rates of both reactions are approximately the same. Thus the rate of polymerisation is determined by stage *1* or *3*, depending on the nature of the anhydride of the *N*-carboxy-α-amino-acid.

This conclusion agrees with earlier observations. The polymerisation of *N*-carboxy-α-amino-acid anhydrides usually is accelerated by increase of temperature [225]. In the polymerisation of sarcosine in nitrobenzene and in acetophenone initiated by sarcosine dimethylamide or poly-(sarcosine dimethylamide) a negative temperature effect was observed [232,233]. According to Heyns' findings [150], the rate of polymerisation in this case is determined not by only one individual reaction but by all the reactions at once.

The removal of carbon dioxide from the growing chain (stage *4*) is reversible. An increase in carbon dioxide pressure slows down the breakdown of the terminal carbamic acid group; this group can be further stabilised by the formation of carbamates with free terminal amino--groups, which reduces their concentration and the rate of polymerisation. For this reason, measurements of the rate of reaction from the carbon dioxide evolved using constant pressure [225] and constant volume [232] apparatus usually differ from one another.

In a homogeneous medium at constant carbon dioxide pressure the rate of polymerisation should also be constant, since the reactivity of the terminal amino-group does not vary with chain growth. Indeed, after the termination of the initiation stage, the polymerisation is a first-order reaction with respect to the anhydride. This was confirmed by Ballard and Bamford for the polymerisation of the anhydrides of the *N*-carboxy-derivatives of DL-leucine, DL-phenylalanine [225], and sarcosine [234], by Breitenbach and Allinger [222] for the polymerisation of the anhydride of *N*-carboxyl-DL-phenylalanine in nitrobenzene, and by Lundberg and Doty [199] for the polymerisation of the anhydride of γ-benzyl *N*-carboxy--L-glutamate in dimethylformamide.

Short chains [235-237] of poly(γ-benzyl L-glutamate) have the random β-conformation in inert solvents, while in the case of long chains the α-spiral conformation is more stable. It might have been expected that during polymerisation the polypeptide chains undergo changes in conformation on attainment of a certain length and that these changes will be reflected in the polymerisation process. Indeed [199,238], the rate of polymerisation of the anhydride of γ-benzyl *N*-carboxy L-glutamate in dioxan, nitrobenzene, dichloroethane, chloroform, and benzene in the presence of n-hexylamine increases approximately fivefold when the growing chains reach a length equivalent to eight structural units (Fig. 11). In the presence of a larger amount of hexylamine ($[M_0]/[I_0] = 4$) the appearance of the second, more rapid stage is not observed, since in this case only short chains are formed, while in the initiation with a polypeptide having $\bar{P}_n = 10$ there is only the rapid stage of chain growth.

It has been demonstrated by infrared spectroscopy that the formation of the poly(γ-benzyl L-glutamate) α-spiral begins on attaining a chain length equivalent to 7–13 units [200], and certainly not less than 5 units [239]. For this reason, Lundberg and Doty attribute the sharp increase in the rate of polymerisation to changes in the conformation of the growing polypeptide chain.

The formation of a new polypeptide fraction in the second stage of the reaction has been convincingly demonstrated by paper chromatography [226] and the measurements of the electric double refraction (Kerr effect) [240]. Additional confirmation that the polymerisation proceeds in two stages was obtained in a study of the polydispersity of poly-(γ-benzyl L-glutamate) [199,203,241]. In dimethylformamide, where only one stage is observed, the polymer exhibits a very narrow molecular weight distribution. In dioxan and chloroform, where the reaction takes place in two stages, the various polymeric chains grow at different rates, depending on their conformation, and give rise to a polypeptide with a very wide molecular weight distribution. The average degree of polymerisation is much higher than the theoretical value, calculated from the relation $\bar{P}_n = [M_0]/[I_0]$, and is in no way related to it.

From a study of the dispersion of optical rotation [242,244], it has been shown that poly(γ-benzyl L-glutamate) has the configuration of a right-hand spiral, while its antipode should have the configuration of a left-hand spiral. If the configuration is important, the rate of polymerisation should depend also on the relative concentrations of the

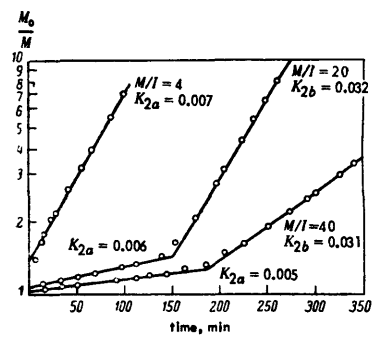


Fig. 11. The rate of polymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate in dioxan at 25°; initial concentration of the monomer $[M_0] = 4\%$; the units of K_2 are litre mole^{-1} s^{-1}.

optical antipodes in the initial mixture. Indeed, the pure optical isomers polymerise twice as fast as the racemic mixture of the D- and L-antipodes. The second stage begins in the latter case much later, which is due to the much later formation of the stable racemic α-spiral [199,245].

When poly(γ-benzyl L-glutamate) is used as the initiator, the polymerisation of the anhydride of the *N*-carboxy--L-derivative proceeds at a high rate and the optical rotation of the system steadily increases with the growth of the α-spirals. In the case of the racemic anhydride the polymerisation proceeds at a rate smaller by a factor of two, but the form of the spirals does not change when D-units are incorporated in it. In the L-polymer (initiator)–D-anhydride system a different relation obtains (Fig. 12). Initially D-units enter into the composition of the right--hand spiral of the L-polymer without changing its form, but as the D-units accumulate inversion of the optical rotation of the system takes place with simultaneous increase of the rate of polymerisation, which is probably due to the transformation of the right-hand α-spiral of the L-polymer into the left-hand α-spiral characteristic of the D-polymer. Comparison of the relative rates of addition of the D- and L-antipodes to the right-hand α-spiral of the L-polymer shows that the L-anhydride is almost five times more reactive than the D-anhydride.

Two stages were also observed in the polymerisation, initiated by n-hexylamine, of the anhydrides of the *N*-carboxy--derivatives of L-leucine in nitrobenzene [199], *O*-benzyloxycarbonyl-L-tyrosine [246], glycine, ϵ-*N*-benzyloxycarbonyl-L--lysine, γ-benzyl L-glutamate, DL-alanine, DL-α-aminobutyric acid, DL-leucine, and DL-valine in dioxan [247]. However, in these cases the polymer is usually precipitated in the form of a gel, the reaction being accelerated simultaneously with the appearance of the new phase.

Ballard and Bamford [248] questioned the existence of two stages in homogeneous polymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate. According to their observations, the reaction is of first order with respect to the anhydride, and has a constant rate whether it is initiated by n-hexylamine or oligopeptides with $\bar{P}_n$ = 4.5 and 15. Doty and Lundberg [249] indicate that the second, rapid stage is observed only in experiments with thoroughly purified anhydrides. Impurities at low concentrations, for example 0.67 mole % of hexylammonium chloride, completely eliminate the second stage. Furthermore, an additive of this kind results in the formation of a polymer with a much lower molecular weight and a much narrower molecular weight distribution.

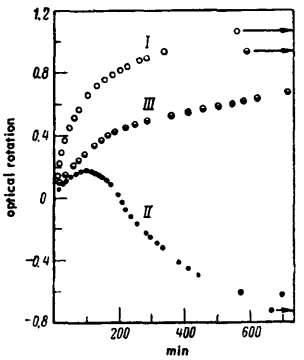


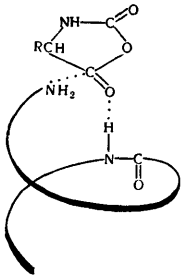
Fig. 12. Variation of the optical rotation of the system during the polymerisation of the anhydrides of γ-benzyl *N*-carboxy-glutamate [I) L-isomer; II) D-isomer; III) DL-isomer] initiated by the L-polymer; solvent — dioxan; temperature 25°; initial concentration 2%; optical pathlength of the cell l = 20 cm.

In the latest papers [234,250] Ballard and Bamford state that in most systems homogeneous conditions do not obtain, since polypeptides are poorly soluble and form a gel even at the trimer and tetramer stage, the rate of polymerisation increasing by a factor of 5–8 at the same time. Further coagulation of the gel into denser formations lowers the rate. Probably this is due to the adsorption of the polymerising anhydride on the polymeric gel. The polymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate, whose polymer does not form true solutions in dioxan according to the above authors, has been assigned to reactions of the same class.

Adsorption can occur also in true solutions as a result of the formation of hydrogen bonds between monomer molecules and the polymeric chain, which probably in fact explains the "chain effect" in polysarcosine [231], poly-*N*-ethylglycine, and poly-*N*-benzylglycine [250], their reactivity increasing with increase in $\bar{P}_n$ in the initiation of the polymerisation of the anhydrides of *N*-carboxy-derivatives with free NH groups. The polymerisation of the anhydride of *N*-carboxysarcosine itself is not autocatalytic in a homogeneous medium, since polysarcosine does not form hydrogen bonds with the monomer [250].

However, the single-stage polymerisation of γ-benzyl *N*-carboxy-L-glutamate in dimethylformamide, in which the polymer is readily soluble and forms α-spirals, contradicts both the hypothesis of Lundberg and Doty that the conformation of the growing chain influences the rate of polymerisation and the hypothesis of Ballard and Bamford that adsorption on polymeric chains takes place.

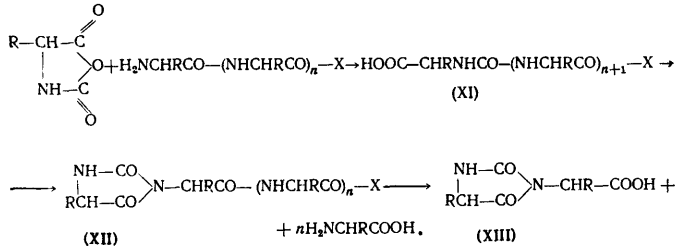
The two hypotheses have been in a way combined in the mechanism proposed by Weingarten [247], according to which activating adsorption of anhydride molecules is possible only at the ends of the growing chains when these have the spiral conformation:



Thus the molecule of the anhydride of the *N*-carboxy--derivative being added assumes a definite orientation even before its reaction with the terminal amino-group. Below yet another attempt will be made to explain the occurrence of the two stages in the polymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate.

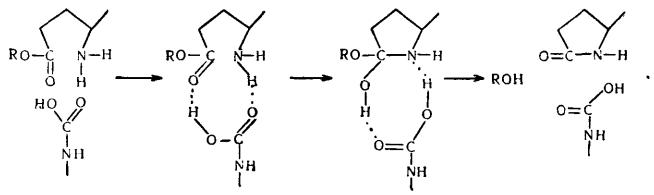
Chain termination. The disappearance of the free amino-group at the end of the polypeptide chain, for any reason, results in the cessation of its growth. The cessation of chain growth occurs as a result of the "erroneous"

addition of the terminal amino-group to the carbonyl group in position 2 of the anhydride ring [192,251,252] with formation of the urea derivative (XI), which is capable of cyclising into the hydantoin derivative (XII). In the acid hydrolysis of polypeptides this derivative may be isolated in the form of the substituted hydantoin-3-acetic acid (XIII).



Possibly the cyclisation takes place only during hydrolysis, which is indicated by the presence of free carboxy--groups in polypeptides [241,251,253].

Chain termination can also occur in the intramolecular interaction of the terminal amino-group with any reactive group in the polymeric chain. Thus in the preparation of poly(γ-benzyl L-glutamate) great importance attaches to the intramolecular cyclisation due to the reaction of the terminal amino-group with the ester group of the same structural unit, benzyl alcohol being eliminated [199,241,254]. Recently it has been shown that the cyclisation is catalysed by weak acids, including carbamic acid, and has the following mechanism [164]:



Intramolecular chain termination as a result of the migration of the acetyl group from the oxygen to the nitrogen was noted in the polymerisation of the anhydride of the *N*-carboxy-derivatives of *O*-acetoxyproline [255] and *O*-acetylserine [257,258].

Polymerisation kinetics. The polymerisation of the anhydrides of *N*-carboxy-derivatives is very convenient for the investigation of the kinetics of the process, the rate of which may be determined both by the titration of the monomer which failed to react [158,247] and from the amount of carbon dioxide evolved during polymerisation. In the latter case more reliable results are obtained at constant carbon dioxide pressure [206,225]. Closed systems of constant volume [187,232,233,259] are less suitable for kinetic studies. A convenient method is one in which the carbon dioxide evolved is swept by dry nitrogen into an absorption vessel where the change in the concentration of sodium hydroxide [222], barium hydroxide [199], or benzylamine [158] is determined conductimetrically [199,222] or by titration [158]. Idelson and Blout [200] studied the kinetics of the polymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate by infrared spectroscopy, which makes it possible to follow the disappearance of the monomer, the formation of the polymer, and the changes in the conformation of the polymeric chain.

In most cases of homogeneous polymerisation with fairly high basicity of the initiator and low rate of chain termination there is direct proportionality between the rate of reaction and the initial concentration of the monomer $[M_0]$ and initiator $[I_0]$. The kinetics of this process were examined theoretically by a group of English workers [225,232] and in a general form by Katchalski and coworkers [260,261]. Three stages of the process are distinguished: initiation (K_1), chain growth (K_2), and chain termination (K_3); the process can take place in one of the following possible ways.

If the rate of initiation is equal to the rate of chain growth ($K_1 = K_2$) and chain termination may be neglected ($K_3 = 0$), the rate of polymerisation is given by

$$-\frac{d[M]}{dt} = \frac{d[CO_2]}{dt} = K_2[I_0][M].$$

These conditions are satisfied by the homogeneous polymerisation of the anhydrides initiated by the amides or esters of amino-acids, peptides, or polypeptides, in solvents where the process takes place in one stage only and at low temperatures when chain termination does not play a significant role. The number-average degree of polymerisation of the polymer formed is defined by the relation $\bar{P}n = ([M_0] - [M])/[I_0]$, or $\bar{P}n = [M_0]/[I_0]$ on completion of polymerisation when $[M] = 0$. The molecular weight distribution should be of the Poisson type, i.e. the polydispersity is low with $\bar{P}_w/\bar{P}_n \simeq 1$.

The actual molecular weight distribution has been determined in only a few cases. It proved to be fairly narrow for polysarcosine [262], poly(γ-benzyl L-glutamate) [199,263], and poly-ϵ-*N*-benzyloxycarbonyl-L-lysine [246], obtained in dimethylformamide.

If the rates of initiation and chain growth differ from one another ($K_1 \neq K_2$), then when the rate of chain termination is low ($K_3 = 0$) the rate of polymerisation is given by the expression

$$-\frac{d[M]}{dt} = \frac{d[CO_2]}{dt} = K_1[I][M] + K_2\sum_{i=1}^{\infty} N_i^*[M],$$

where $[I]$ represents the concentration of the initiator at any instant, and N_i^* the concentration of growing polymer chains containing at a given instant the following number of i-units: $[I] + \sum_{i=1}^{\infty} N_i^* = [I_0]$. At the early stages of the process when $[I_0] \simeq [I] \gg \sum_{i=1}^{\infty} N_i^*$, the overall rate of reaction is determined mainly by the first term of the equation, and on completion of polymerisation, when $[I] \ll \sum_{i=1}^{\infty} N_i^* \simeq [I_0]$, by the second term. Thus in this case polymerisation is of first order with respect to the anhydride and the rate of evolution of carbon dioxide is determined by the constants K_1 at the beginning and K_2 at the end of polymerisation. If $K_1 > K_2$, which occurs when the initiators are strong bases, the initiation process terminates rapidly and the rate of chain growth remains constant throughout the process. The molecular weight distribution of the polymer is very narrow and $\bar{P}n = [M_0]/[I_0]$. When weak initiators are used, with $K_1 < K_2$, the initiation process is protracted and the overall rate of the reaction increases with increase in the number of new chains, and the polymer formed is polydisperse. $\bar{P}n = [M_0]/[I_0]$ obtains only in the case where the entire initiator is able to react before the end of polymerisation.

If in the case of rapid initiation ($K_1 > K_2$, $K_3 \simeq 0$) two stages are observed in the chain propagation process (K_{2a} and K_{2b}), the transition from K_{2a} to K_{2b} being completed

after the attainment of a chain length equivalent to s-units, the rate of the process is given by the expression

$$-\frac{d[M]}{dt}=\frac{d[CO_2]}{dt}=K_1[I][M]+K_{2a}\sum_{i=1}^{i=s}N_i^*[M]+K_{2b}\sum_{i=s}^{\infty}N_i^*[M].$$

This case was examined by Katchalski[246] on the basis of the kinetic data obtained by Lundberg and Doty[199] for two-stage polymerisation of the anhydride of γ-benzyl N-carboxy-L-glutamate in dioxan. Similarly to the preceding case, the polymerisation is again of first order with respect to the anhydride, its rate being determined mainly by the constant K_{2a} at the beginning and K_{2b} at the end: $-d[M]/dt = K_2[I_0]/[M]$. The polymer fractions with a degree of polymerisation less than s exhibit the Poisson distribution. The fractions with $\overline{P}_n > s$ are more polydisperse the greater the difference between K_{2a} and K_{2b}. On the basis of mathematical analysis, the authors believe that the number of β-chains diminishes during the polymerisation at a slower rate the greater the value of s, and the weight fraction of the β-polymer falls after reaching a maximum proportional to s. It is reasonable that the time of transition from K_{2a} to K_{2b} depends only on $[M_0]$ and not on $[I_0]$, since the rate of formation of the polymer with $\overline{P}_n = s$ depends on the concentration of the monomer $[M_0]$ and not on the number of chains $[I_0]$.

However, the relation observed by Doty and Blout[199,200] between $[M_0]$ and the time of transition from K_{2a} to K_{2b} is the opposite of that which would be expected on the basis of Katchalski's conclusions (Fig. 11). Furthermore, in one of his latest papers Katchalski[256] notes that the polydispersity found by Doty ($\overline{P}_w/\overline{P}_n = 8.5$) is much greater than the theoretical value. Thus the conclusions derived from an analysis of the polymerisation kinetics on the basis of the hypothesis of Doty and Lundberg, that chain conformation influences the rate of chain growth, is in poor agreement with experiment. On the other hand, no conclusion can be reached about the hypothesis of Ballard and Bamford that the adsorption of the monomer on polymer chains affects the rate of polymerisation, since the example examined by Katchalski assumes that K_{2a} and K_{2b} are constant, a possibility which is excluded in Ballard's hypothesis.

If in the case of a high rate of initiation and a single-stage process ($K_1 > K_2$) some of the polymerising chains are terminated ($K_3 \neq 0$) as a result of a bimolecular reaction with the anhydride, the amount of carbon dioxide evolved is less than that of the anhydride which has reacted ($[M_0] - [M]$). The difference $[M_0] - [M] - [CO_2]$ corresponds to the amount of terminal carboxy-groups. Katchalski[260,261] showed that in this case the molecular weight distribution of the growing chains remains of the Poisson type, but the polymer fraction with terminal carboxy-groups is polydisperse with $\overline{P}_w/\overline{P}_n \simeq 2$. The maximum attainable degree of polymerisation of the polymer as a whole is then $\overline{P}_n = K_2/K_3$.

This case is close to real polymerisation conditions. The chain termination reaction, which is rare at low temperatures (one reaction for 300 addition reactions)[199,241] plays a considerable role at elevated temperatures, since the activation energy for chain termination is somewhat higher than that for chain propagation, which in the case of γ-benzyl L-glutamate is only 6.6 kcal mole^{-1}.[199,200]

The kinetics of the polymerisation of the anhydrides of the N-carboxy-derivatives of trifunctional acids, for which intramolecular chain termination is possible, have not been examined theoretically.

TABLE 15. Rate constants $K_{2(a)b}$ (litre mole^{-1} s^{-1}) for the polymerisation of the anhydrides of various N-carboxy--α-amino-acids.

Anhydride of N-carboxy-derivative of	Solvent	Initiator	t, °C	$10^2K_{2(a)b}$	References
Glycine	dioxan	p-bromoaniline	37	18	227
Sarcosine	benzene	n-hexylamine	25	(12)60	234
L-Alanine	»	»	25	(6.3)54	234
»	nitrobenzene	»	25	(4.8)26.4	234
L-Leucine	»	»	25	(1.3)9	199
Phenylalanine	»	»	25	(1.6)	234
»	benzene	»	25	(5)	234
γ-Benzyl L-glutamate	dioxan	»	34	2.5–5.2	200
Ditto	dichloroethane	»	25	(1.5)14	199
»	dimethylformamide	»	25	5.7	199
»	»	»	0	1.9	199
»	»	»	40	8.7	199
»	dioxan	$NaOCH_3$	34	430–630	277
γ-Benzyl DL-glutamate	»	n-hexylamine	25	(0.4)1.5	199
Ditto	dimethylformamide	»	25	2.2	199
γ-Methyl L-glutamate	chloroform	CH_3COOH	30	(0.35)2.9	292

Table 15 lists the latest data on the rates of polymerisation of the anhydrides of N-carboxy-derivatives, from which it follows that the rate of polymerisation is a maximum in the case of glycine and proline. Racemates polymerise more slowly than enantiomers. Non-polar solvents, which cannot form hydrogen bonds, accelerate the reaction[199].

The determinations of the rates of polymerisation are not very accurate because of the difficulty of purifying the anhydrides of N-carboxy-derivatives and the solvents, and because of failure to achieve the conditions of a homogeneous reaction[247]; the fact that the reaction is homogeneous is established with difficulty by a visual procedure (cf. Refs. 199 and 250). Furthermore, because the reaction of the anhydrides of N-carboxy-derivatives with strong amines proceeds in different ways, the true concentration of growing chains is only approximately equal to the initial concentration of the initiator. When the process takes place in two stages, the constant K_{2b} is much too low, since not all the growing chains attain a length necessary for the acceleration of the reaction. Finally, polymerisation initiated by primary and secondary amines must proceed in several ways simultaneously. Otherwise it would have to be of first order with respect to the initiator and the rate of chain propagation would be independent of the nature of the initiator.

In fact K_2 does depend on the initiator. Thus diethylamine leads as a rule to polymerisation twice as fast as that given by hexylamine[247]. Furthermore, the order of the reaction with respect to the initiator is sometimes other than unity. In the polymerisation of the anhydrides of the N-carboxy-derivatives of DL-valine, DL-leucine, ϵ-N-benzyloxycarbonyl-DL-lysine, and DL-α-aminobutyric acid in dioxan a fractional reaction order was found[247]. In the polymerisation of the anhydride of N-carboxysarcosine Heyns and Schultze[150] found the reaction to be of second order with respect to the initiator, and later it was shown that the rate is proportional to the expression

$$a[I_0]+b[I_0]^2 \quad ,$$

where a and b are constants[232,247].

In an investigation of the two-stage polymerisation of the anhydride of γ-benzyl N-carboxyl-L-glutamate the slow stage was found to be of first order with respect to the

anhydride and its rate proved to be independent of the initiator. On the other hand, the second, rapid stage was of fractional order (3/2) with respect to the anhydride and its rate did depend on the initiator [228].

The authors propose the following mechanism for chain propagation which explains the peculiarities of this process:

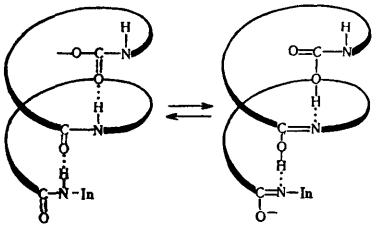
$$P_n\text{—}NH_2 + M \xrightarrow{K_{2a}} P_n\text{—}NHCOCHRNHCOOH, \quad (c)$$

$$P_n\text{—}NHCOCHRNHCOOH \xrightarrow{K_4} P_n\text{—}NHCOCHRNH_2 + CO_2, \quad (d)$$

$$P_n\text{—}NH_2 + P_m\text{—}NHCOOH \xrightarrow{K} P_n\overset{+}{N}H_3 + P_mNHCOO^-, \quad (e)$$

$$P_mNHCOO^- + M \xrightarrow{K_{2b}} P_mNHCOCHRNHCOO^- + CO_2. \quad (f)$$

Chain propagation can proceed according to the generally accepted mechanisms (c)–(d) with a rate constant K_{2a} or by reaction (f) with a higher rate constant K_{2b}. Usually the contribution of reaction (f) is small because of the instability of carbamic acid and the resulting low concentration of the carbamate anion. However, on formation of the α-spiral the stability of the anion may be significantly increased because of the distribution of the charge throughout the spiral:



As a result, the initiator can influence the stability of the carbamate anion and hence the rate of polymerisation. The rate of reaction (f) is defined by the equation

$$\frac{d[M]}{dt} = K_{2b}\left(\frac{K_{2a}}{K_4}\right)^{\frac{1}{2}} K^{\frac{1}{2}} [I_0]\,[M]^{\frac{3}{2}},$$

i.e. the reaction is of order 3/2 with respect to the anhydride, which agrees with experiment.

The mechanism proposed constitutes a very interesting attempt to account for the effect of spiral conformation and the initiator on the rate of polymerisation. However, we feel bound to point out that according to this mechanism secondary amines should not affect the reactivity of the terminal amino-group of the growing chain, since they are incapable of participating in the suggested system of hydrogen bonds.

Bamford and Block [264] proposed another mechanism for the effect of the initiator on the rate of polymerisation, which will be examined below.

C. Initiation with water. Polymerisation in the presence of water proceeds similarly as the polymerisation initiated by amines [265]. However, the initiation stage is slower because of the lower basicity of water, and the ionisation of free carboxy- and amino-groups in the system greatly slows down chain growth, particularly in the first polymerisation stage [224,266–270]. The use of water as the initiator is of interest for the preparation of polypeptides with terminal carboxy- and amino-groups capable of further condensation, for example by the carbodiimide method [271].

In the case of initiation by water side-reactions are possible. The polyglycine obtained in this way contains up to 2% of 2,5-dioxopiperazine [253]. Polymerisation in polar solvents, for example dimethylformamide, probably takes place by a different mechanism and leads to considerable amounts of hydantoin derivatives [207,272].

In many cases water can be used not only as the initiator but also as the polymerisation medium [174]. In recent years the dependence of the rate of polymerisation of various anhydrides of *N*-carboxy-derivatives on the pH of the medium has been studied in detail [163,269,273–275].

D. Initiation with strong bases. Sodium hydroxide [276], sodium methoxide, sodium borohydride [203], and triphenylmethylsodium [277], which have been proposed by Blout and coworkers as the initiators of the polymerisation of the anhydrides of *N*-carboxy-derivatives, yield exclusively high-molecular-weight polypeptides [poly(γ-benzyl L-glutamate, M = 600 000; poly-ϵ-*N*-benzyloxycarbonyl--L-lysine, M = 2 000 000], which are of low dispersity and are free of 2,5-dioxopiperazine and other cyclic compounds [278,279]. A detailed study of this process [203,277,280,281] has shown that polypeptides with the maximum molecular weights are formed in solvents such as dioxan, chloroform, and benzene at temperatures below 30° and monomer concentrations below 4%. In nitrobenzene the rate of polymerisation is smaller by a factor of almost 30 and the polymer has a much lower molecular weight. There is no proportionality between the degree of polymerisation and the ratio $[M_0]/[I_0]$, the molecular weight of the polymer being always higher than the calculated value [203,281].

The rate of polymerisation and the molecular weight of polymers increase in the series lithium, sodium, potassium, and caesium methoxides [280]. The polymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate in dioxan at 34° proceeds after a brief autocatalytic period as a first--order reaction with respect to the monomer at a rate one hundred times greater (K_2 = 4.3–6.3 litre mole^{-1} s^{-1}) than in the case of initiation by primary or secondary amines [280]. Addition to the reaction system of fresh portions of the anhydride of the *N*-carboxy-derivative does not increase the

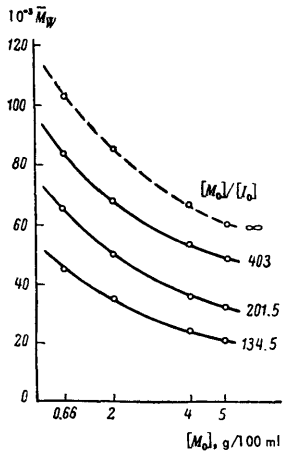


Fig. 13. Dependence of the molecular weight $\bar{M}_w$ of poly-DL-phenylalanine on the initial monomer concentration $[M_0]$ for various values of $[M_0]/[I_0]$.

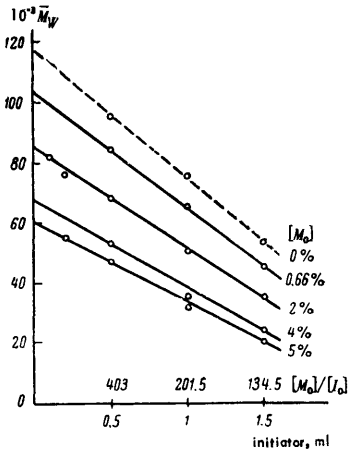
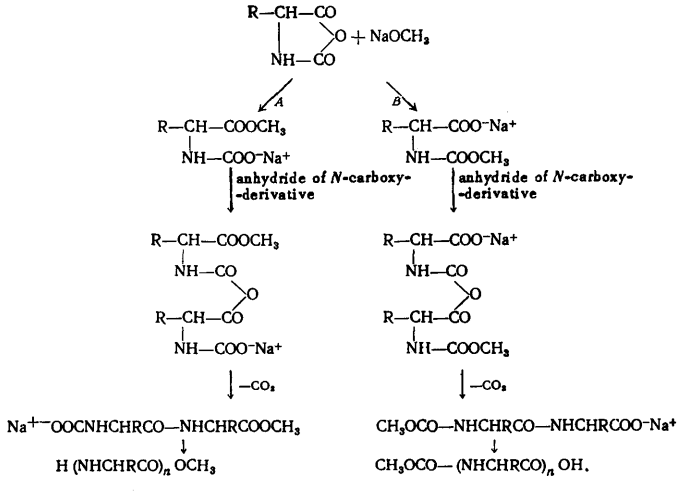


Fig. 14. Dependence of the molecular weight $\bar{M}_W$ of poly-DL-phenylalanine on the ratio $[M_0]/[I_0]$ for various initial monomer concentrations $[M_0]$.

molecular weight of the polymer. The replacement of the L-anhydride by the racemate lowers the rate of polymerisation by a factor of seventeen and the molecular weight of the polymer by a factor of about five.

The above features of the process have also been found for the neutral amino-acid DL-phenylalanine[279]. Figs. 13 and 14 show the experimental dependence of the molecular weight $\bar{M}_W$ of the polypeptide on the concentration of the anhydride $[M_0]$ and the ratio $[M_0]/[I_0]$. The polymerisation was carried out at 40° in chloroform in the presence of sodium methoxide. The dashed lines, corresponding to $[M_0] = 0$ and $[M_0]/[I_0] = \infty$ (0 ml of initiator) were obtained by extrapolation. The molecular weights were found from the light scattering.

These results led Blout and Idelson[280] to the hypothesis that the polymerisation in this case has an ionic mechanism:



The growth of the polymeric chain proceeds by successive addition of the polymeric anions to the anhydride of the *N*-carboxy-derivative either at the carbonyl in position 5 (pathway *A*) or at the carbonyl in position 2 (pathway *B*) with formation of mixed anhydrides which readily eliminate carbon dioxide. Chain termination occurs when the addition at the carbonyl in position 5 is followed by the addition at the second carbonyl group and conversely:

$$Na^+{-}OOC(NHCHRCO)_n OCH_3 \xrightarrow{\text{anhydride of } N\text{-carboxy-derivative}}$$

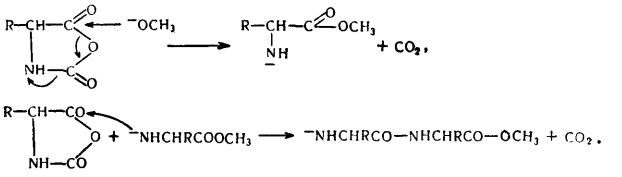
$$\rightarrow Na^+{-}OOCCHR{-}NHCOOCO(NHCHRCO)_n OCH_3 \rightarrow Na^+{-}OOCCHRNHCO(NHCHRCO)_n OCH_3 \rightarrow$$

$$\rightarrow CH_3OCO(NHCHRCO)_n O{-}Na^+ \rightarrow CH_3OCO(NHCHRCO)_n OCOCHRNHCOO^-Na^+ \rightarrow$$

$$\rightarrow CH_3OCO(NHCHRCO)_n OCOCHRNH_2.$$

In the first case the polymer should contain urea and in the second anhydride and urethane groups.

A more probable mechanism[282] for chain propagation[280], also proposed by the same workers, is one in which the participation of an amine anion is assumed, and the elimination of carbon dioxide is attributed to simple electron pair transfers:



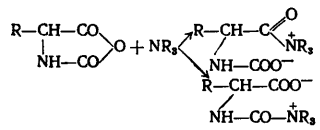
E. Initiation with tertiary amines. Tertiary amines (pyridine[174,182,224,283-285], α-picoline, 2,6-lutidine[182], triethylamine[103,259,286,287], tri-n-butylamine[259,285,288], and trimethylamine[289]) are effective initiators for the polymerisation of the anhydrides of *N*-carboxy-derivatives with a free NH group. However, the resulting polypeptides contain some substituted hydantoin-3-acetic acid[207,283].

As regards the anhydrides of *N*-substituted *N*-carboxy--derivatives, there is no single view concerning their ability to polymerise under the action of tertiary amines. In contrast to the anhydride of *N*-carboxy-*N*-cyclohexylglycine[284], the anhydride of *N*-carboxy-*N*-phenylglycine does not polymerise in pyridine even at an elevated temperature[174,175]. The anhydride of *N*-carboxysarcosine polymerises at room temperature in pyridine[175,283] even when it is most thoroughly purified by means of phenylmagnesium bromide[284]. The anhydrides of the *N*-carboxy--derivatives of L-proline[141,290], L-hydroxyproline, and *O*-(*p*-toluenesulphonyl)-L-hydroxyproline[290] also polymerise readily in pyridine. Nevertheless, Ballard and Bamford[182,206,259,285] believe that the polymerisation of sarcosine and proline and their derivatives is due to impurities and that after thorough purification it is possible to reduce to a minimum the initial rate of polymerisation[259].

Tertiary amines give rise to rapid polymerisation of the anhydrides of *N*-carboxy-derivatives in organic solvents even in the case of high $[M_0]/[I_0]$ ratios, yielding high--molecular-weight polypeptides, the degree of polymerisation of which is independent of the $[M_0]/[I_0]$ ratio[203]. These features resemble in many ways the polymerisation initiated by strong bases.

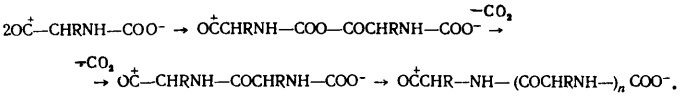
The polypeptides obtained in an anhydrous medium with tertiary amines as the initiators cannot have terminal amino- and carboxy-groups, i.e. they must have a cyclic structure. Thorough analysis of polysarcosine (M = 22 000) and poly-*N*-cyclohexylglycine, prepared in dry pyridine, showed that these polymers have virtually no end-groups[284]. Cyclic dipeptides, substituted 2,5-dioxopiperazines, have been isolated, the yield reaching 35% in the case of poly--*N*-cyclohexylglycine. According to Wieland[291], in this

case the anhydride ring is opened by the tertiary amine with formation of a zwitter-ion:

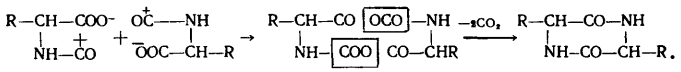


The formation of polypeptides, 2,5-dioxopiperazines and substituted hydantoin-3-acetic acid can be explained by different possibilities of combining the zwitter-ions.

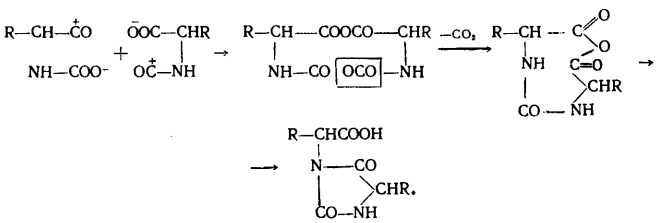
Formation of polypeptide:



Formation of 2,5-dioxopiperazine:

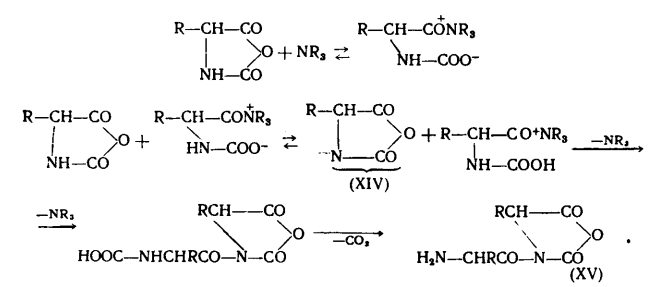


Formation of substituted hydantoin-3-acetic acid:

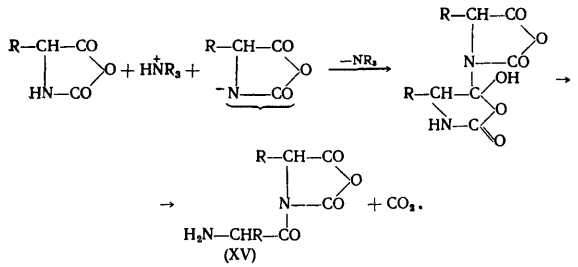


The rapid polymerisation of the anhydrides of *N*-carboxy-derivatives in organic solvents in the presence of catalytic amounts of tertiary amines[203] shows, however, that the growth of the polypeptide chain proceeds mainly not as a result of the interaction of two zwitter-ions but as a result of their reaction with the anhydrides of *N*-carboxy--derivatives.

The mechanism proposed by Ballard and Bamford[182,259,285] for the reaction between the anhydrides of *N*-carboxy--derivatives and tertiary amines excludes the possibility of the polymerisation of the anhydrides of *N*-substituted *N*-carboxy-derivatives, since the primary products of the reaction are believed to be the anhydride anion (XIV) and 3-(α-aminoacyl)-2,5-dioxooxazolidine (XV):

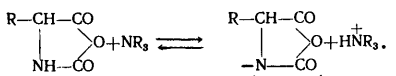


Subsequently the anion (XIV) can also react with a molecule of the anhydride by the mechanism



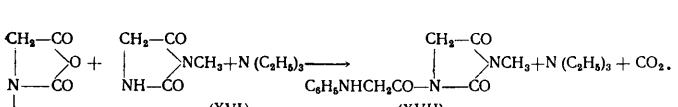
Compound (XV) initiates chain growth both at the amine end and at the *N*-carboxy-derivative anhydride end.

However, measurements of the rate of polymerisation of the anhydrides of the *N*-carboxy-derivatives of γ-ethyl L-glutamate in dimethylformamide and of DL-phenylalanine in nitrobenzene in the presence of pyridine, α-picoline, and 2,6-lutidine as the initiators[182] showed that the anion (XIV) does not form as a result of the nucleophilic attack by the tertiary amine on the carbonyl group in position 5 but as a result of direct attack by the amide hydrogen (cf. Ref. 181):



Otherwise the rate of reaction of the tertiary amines with the anhydrides of the *N*-carboxy-derivatives should decrease in the series pyridine, α-picoline, 2,6-lutidine due to increasing steric hindrance, while experiment showed that the rate actually increases in this series because of the increasing basicity of the amines.

Chain termination occurs also as a result of intramolecular cyclisation of the bifunctional polymeric chain or as a result of deactivation of the terminal amino-group. The formation of substituted hydantoin-3-acetic acid is explained by a rearrangement of compound (XV). In the authors' view[259] the mechanism proposed is proved by the fact that the rapid polymerisation of the anhydride of *N*-carboxy-sarcosine proceeds only in the presence of 3-methylhydantoin (XVI), an analogue of the anhydride of the *N*-carboxy--derivative not substituted at the nitrogen atom, and also by the fact that it proved possible to isolate 3-methyl-1--(*N*-phenylglycyl)hydantoin (XVII) from the products of the reaction of the anhydride of *N*-carboxy-*N*-phenylglycine, triethylamine, and 3-methylhydantoin:



As already pointed out, the suggested mechanism excludes the possibility of the polymerisation of *N*-substituted anhydrides, which cannot be regarded as completely proved. Furthermore, it entirely fails to account for the extremely rapid rate of polymerisation, which is comparable with the rate of ionic polymerisation. The chain growth of the anhydride end cannot increase significantly the rate of polymerisation, since it involves interaction either with

activated molecules of the anhydride of the *N*-carboxy--derivative, the concentration of which is extremely low, or with terminal amino-groups, which would reduce the concentration of the active centres for chain propagation.

It has been shown[264] that the polymerisation in the presence of secondary and primary amines also frequently proceeds by the mechanism characteristic of the initiation by tertiary amines. Blout[203] observed a considerable similarity between the polymerisations of the anhydride of γ-benzyl *N*-carboxy-L-glutamate initiated by diethylamine and triethylamine. In both cases the polymerisation was rapid and the molecular weight of the polymer was independent of the $[M_0]/[I_0]$ ratio.

Bamford found (Fig. 15) that the polymerisation of the anhydrides of the *N*-carboxy-derivatives of sarcosine and γ-ethyl L-glutamate in the presence of n-hexylamine and diisopropylamine takes a normal course only when initiated by n-hexylamine (curves *B* and *C*). Because of the considerable steric hindrance caused by the isopropyl groups, the initiation of the polymerisation of sarcosine by diisopropylamine is slow and the reaction becomes accelerated only as active terminal amino-groups appear in the growing chain (curve *D*). It would appear that this should also hold for other anhydrides. However, in the case of the anhydride of the *N*-carboxy-derivative not substituted at the nitrogen atom (curve *A*) the sterically hindered diisopropylamine gives rise to much more rapid polymerisation than does hexylamine, which can be explained only by the preferential occurrence of the process involving the mechanism characteristic of tertiary amines. This conclusion is also confirmed by the analysis of the molecular weights of the polymers. Whereas poly(γ-ethyl L-glutamate) obtained in the presence of hexylamine has $\overline{P}_n = 60$, in the initiation with diisopropylamine $\overline{P}_n$ reaches 150 under the same polymerisation conditions with $[M_0]/[I_0] = 15$. It is interesting that in the latter case the amount of carbon dioxide evolved is much smaller than the theoretical value.

F. Initiation with salts. Inorganic salts were proposed by Ballard as the initiators of the polymerisation of the anhydrides of *N*-carboxy-derivatives as early as 1954 [206,207] but did not find wide application, since the resulting polypeptides contained up to 30–40% of hydantoin derivatives. However, this type of initiation is very interesting from the chemical point of view, because it is believed that there is considerable similarity in the mechanisms of the reaction of the anhydrides of *N*-carboxy--derivatives with salts and tertiary amines[207,259].

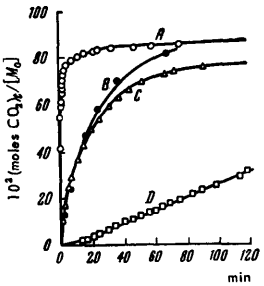
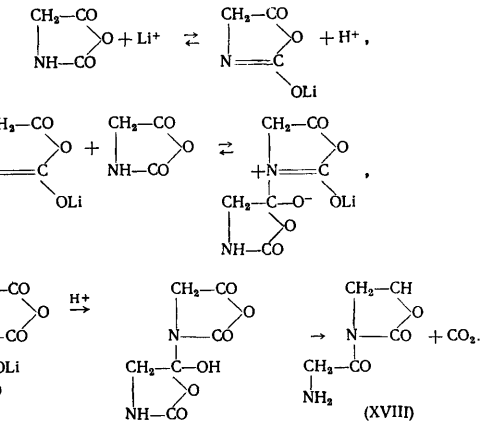
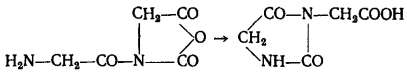


Fig. 15. Rate of evolution of carbon dioxide in different systems in diethylformamide solution at 25°:
A) anhydride of γ-ethyl *N*-carboxy-L-glutamate–diisopropylamine; *B*) anhydride of *N*-carboxysarcosine–n-hexylamine; *C*) anhydride of γ-ethyl *N*-carboxy-L-glutamate–n-hexylamine; *D*) anhydride of *N*-carboxysarcosine–diisopropylamine; $[M_0] = 0.224$ M; $[I_0] = 0.015$ M.

The following mechanism has been proposed for the initiation of polymerisation:

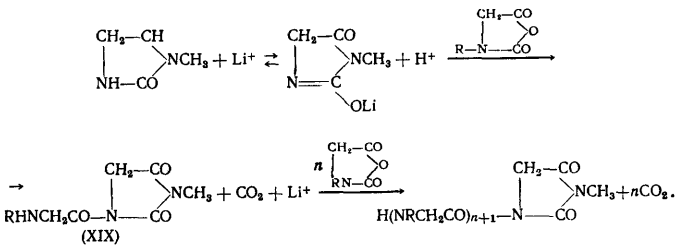


An intermediate compound, 3-aminoacetyl-2,5-dioxooxazolidine (XVIII), is formed and initiates chain growth or is converted into hydantoin-3-acetic acid:

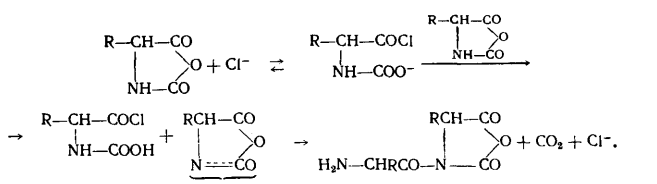


The polypeptide chains have reactive end-groups and can react with one another or form macro-rings.

The anhydrides of *N*-substituted *N*-carboxy-derivatives do not react with salts, but they readily polymerise in the presence of 3-methylhydantoin:



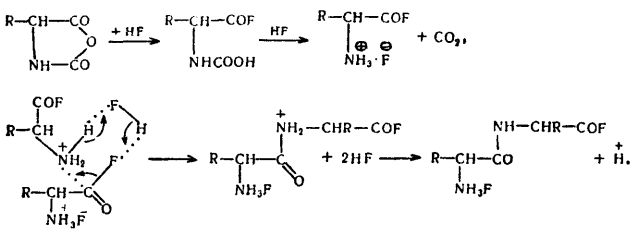
The first chain unit, 3-methyl-1-(*N*-phenylglycyl)hydantoin (XIX) was isolated from the products of the reaction of the anhydride of *N*-carboxy-*N*-phenylglycine, 3-methylhydantoin, and lithium chloride[259]. Later it was found that lithium perchlorate does not initiate the polymerisation of the anhydrides[250], which means that the active component of lithium chloride is not the cation Li^+ but the anion Cl^-.[259]



G. Initiation with acids. Acids usually inhibit the polymerisation of the anhydrides of *N*-carboxy-derivatives. However, the anhydride of γ-methyl *N*-carboxy-L--glutamate rapidly polymerises in chloroform in the presence of acetic acid[292]. With $[M_0]/[I_0] = 2$ the reaction is initially slow ($K_{2a} = 0.0035$), but after 30 min the rate increases sharply ($K_{2b} = 0.029$) and the polymerisation terminates in 2 h with formation of a polypeptide having $\overline{P}_n = 500-600$. The increase in rate corresponds to $\overline{P}_n = 50-60$, which the authors of this investigation attribute to the gel effect.

It is interesting that in dioxan polymerisation does not take place, the addition of 10% of dioxan to chloroform causing the appearance of an induction period (70 min). In the pure acid tripeptides are formed slowly. Acylated products and 2,5-dioxopiperazines have not been detected in the reaction products. The polymerisation of the anhydrides of *N*-carboxy-derivatives in chloroform is also induced by a number of other acids[201].

H. Polymerisation in anhydrous hydrogen fluoride. Anhydrous hydrogen fluoride readily dissolves the anhydrides of *N*-carboxy-α-amino-acids and polypeptides[293]. An attempt was made to use it as a polymerisation medium[294]; the polymerisation then proceeds without additional initiators but involves the racemisation of the amino-acids and the formation of considerable amounts of 2,5-dioxopiperazines. The degree of polymerisation reaches 25–30. The following mechanism has been suggested for this reaction:



I. Initiation with organometallic compounds. The polymerisation of the anhydride of *N*-carboxy-DL-alanine in tetrahydrofuran in the presence of a number of organometallic compounds was investigated recently[295]. Diethylcadmium and triethylaluminium proved to be the most effective compounds. Diethylzinc, phenylmagnesium bromide, and butyllithium were less effective, and tributylboron did not initiate the polymerisation at all. In contrast to other polymers, the polyalanine obtained in the presence of triethylaluminium was insoluble in water. Since poly-D-alanine and poly-L-alanine are also insoluble in water, the authors of this investigation suggested that triethylaluminium causes stereospecific polymerisation of the anhydride of the *N*-carboxy-derivative.

A similar property is shown by aluminium isopropoxide and by catalytic systems consisting of triethylaluminium and methanol or borneol, although in this case the insoluble fraction forms only part of the product. On the other hand, mixtures of diethylzinc with methanol and water and of diethylcadmium with methanol give only the water-soluble poly-DL-alanine.

When the optically active (+)-borneol and (-)-menthol were used as initiators, polyalanine exhibiting optical activity in solution was obtained, but the polymerisation of the monomer was incomplete.

4. Copolymerisation of the Anhydrides of *N*-Carboxy-α--amino-Acids

The available methods for the synthesis of the copolymers of α-amino-acids starting with the anhydrides of the *N*-carboxy-derivatives do not permit the preparation of copolypeptides with a specified sequence of the amino-acid residues. Usually the synthesis of copolypeptides reduces to the polymerisation in the solid state or in solution of a mixture of the anhydrides of the *N*-carboxy-derivatives using as the initiators the same compounds as in homopolymerisation, i.e. primary, secondary, and tertiary amines, sodium methoxide, amino-acid amides, polypeptides with free amino-groups, water, etc.

It is quite reasonable that copolymerisation should be a more complex process than the polymerisation of a pure *N*-carboxy-α-amino-acid anhydride and that it should have a number of specific features. Most of the published studies on copolymerisation were undertaken in order to elucidate the effect of the polypeptide composition on the conformation of macromolecules in solution; less attention was given to problems of the kinetics and mechanism of the reaction. For this reason, a considerable part of these investigations will be examined very briefly in this Section.

Katchalski and Sela[5] considered the copolymerisation of two anhydrides of *N*-carboxy-α-amino-acids as a system of the following reactions:

$$\cdots A + A \xrightarrow{K_1} \cdots A,$$
$$\cdots A + B \xrightarrow{K_2} \cdots B,$$
$$\cdots B + A \xrightarrow{K_3} \cdots A,$$
$$\cdots B + B \xrightarrow{K_4} \cdots B,$$

where A and B are monomers, ...A and ....B the growing peptide chains containing the terminal amino-group of the amino-acids A or B and K_1, K_2, K_3, and K_4 are the rate constants. The anhydrides of *N*-carboxy-derivatives of various amino-acids greatly differ in their reactivity and therefore the above reactions can proceed at different rates. However, Shalitin and Katchalski[319] found that the rates of copolymerisation are determined by the nature of the monomer and not of the terminal amino-acid residue of the growing chain, and therefore $K_1 = K_3$ and $K_2 = K_4$. It has been found that the rates of copolymerisation with diethylamine as the initiator, of the anhydrides of the *N*-carboxy-derivatives of γ-benzyl L-glutamate and ε-*N*-benzyloxycarbonyl-L-lysine in dimethylformamide, which is a good solvent both for homopolymers and copolymers at 25°, are equal to the sum of the rates of polymerisation of the individual monomers (Figs. 16 and 17).

On the basis of the data obtained, the rate of copolymerisation $-d[G + L]/dt$ can be represented thus:

$$-\frac{d[G+L]}{dt} = K_G[I_0][G] + K_L[I_0][L], \quad (1)$$

or

$$-\frac{d[G+L]}{dt} = K_{cop}[I_0][G+L],$$

where

$$K_{cop} = \frac{K_G[G] + K_L[L]}{[G] + [L]}. \quad (2)$$

Since K_G is greater than K_L, the anhydrides of γ-benzyl *N*-carboxy-L-glutamate (*G*) will be consumed more rapidly

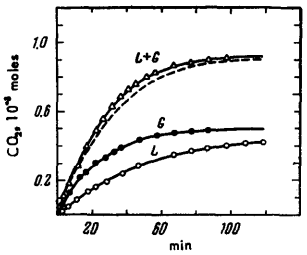


Fig. 16. Polymerisation of the anhydrides of the *N*-carboxy-derivatives of ϵ-*N*-benzyloxycarbonyl-L-lysine (*L*) and γ-benzyl L-glutamate (*G*) and their mixture (*G* + *L*) in dimethylformamide at 25° with diethylamine as the initiator; the dashed curve represents the sum of curves *L* and *G*.

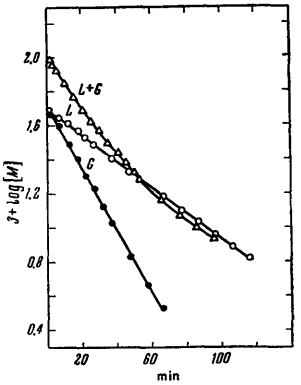


Fig. 17. Consumption of the monomers *L* and *G* as a function of time; [*M*] is the molar concentration of the monomer which has not reacted.

during copolymerisation than the anhydride of ϵ-*N*-benzyloxycarbonyl-α-*N*-carboxy-L-lysine (*L*). For this reason, the reaction mixture at the end of the process will contain only traces of the first anhydride and relatively large amounts of the second, and K_{cop} will be virtually equal to K_L. The time variation of the composition of the copolymer formed is clearly shown in the electrophoretic pattern obtained in phosphate buffer at pH 7.8 for glutamic acid, lysine, and the copolymers of glutamic acid and lysine in the initial and final stages of copolymerisation (Fig. 18).

It is seen that the copolymers isolated at the beginning of the reaction were negatively charged and migrated towards the anode, like glutamic acid, while those isolated at the end of the reaction were positively charged and migrated towards the cathode, like lysine. The copolymers isolated after 25, 50, and 93% of conversion of the monomers contained glutamic acid and lysine residues in molar ratios of 3.0, 1.3, and 0.22 respectively.

The hypothesis concerning the mechanism of copolymerisation made it possible to derive an equation for the composition of the resulting copolymer as a function of

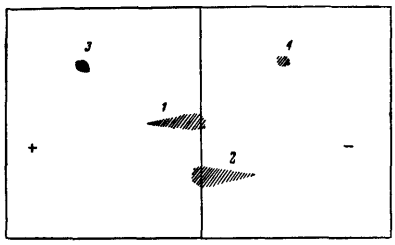


Fig. 18. Electrophoretic pattern of the copolymer of lysine and glutamic acid:
1) copolymer after 20% conversion; 2) copolymer after completion of reaction; 3) glutamic acid; 4) lysine.

time *t* and the initial composition of the reaction mixture $[G_0]/[L_0]$:

$$\frac{[G]_x}{[L]_x} = \frac{[G_0]}{[L_0]} \frac{1-\exp(K_G[I_0])t}{1-\exp(K_L[I])t}, \qquad (3)$$

where $[G]_x/[L]_x$ is the ratio of the glutamic acid and lysine residues in the copolymer.

Investigation of the copolymerisation of other anhydrides of *N*-carboxy-α-amino-acids (L-phenylalanine and ϵ-*N*-benzyloxycarbonyl-L-lysine, L-phenylalanine and γ-benzyl L-glutamate, glycine and ϵ-*N*-benzyloxycarbonyl-L-lysine, ϵ-*N*-benzyloxycarbonyl-L-lysine and DL-alanine, and γ-benzyl L-glutamate and DL-alanine) in dimethylformamide with diethylamine as the initiator established that the copolymerisation of these monomeric pairs proceeds similarly to the copolymerisation of the anhydrides of the *N*-carboxy-derivatives of γ-benzyl L-glutamate and ϵ-*N*-benzyloxycarbonyl-L-lysine, i.e. the rates of copolymerisation are equal to the sum of the rates of polymerisation of the corresponding individual monomers. The resulting copolypeptides had a molecular weight distribution close to the molecular weight distribution of the corresponding homopolymers.

A number of copolypeptides with different contents of L-glutamic acid, L-lysine, L-tyrosine, and L-phenylalanine have been synthesised[320] from the anhydrides of the corresponding N-carboxy-derivatives in absolute benzene at 25° using sodium methoxide as the initiator ($[M_0]/[I_0]$ = = 400). The molecular weights of the resulting copolymers were in the range 50 000–100 000. On the basic of kinetic data, the authors concluded that up to 90% conversion the copolymerisation is of first order, the reaction order departing from unity subsequently. Table 16 presents data on the composition of the initial reaction mixture and the copolypeptides.

A number of copolymers with *M* = 30 000–550 000 containing different amounts of L-glutamic acid, L-lysine, L-tyrosine, L-phenylalanine, and L-alanine were prepared later[321,322]. Blout and Idelson[323] obtained four high-molecular-weight copolypeptides of L-lysine and L-glutamic acid with a disordered structure and different molar ratios of the components (7 : 3, 6 : 4, 5 : 5, and 4 : 6) from the anhydrides of the *N*-carboxy-derivatives of γ-benzyl L-glutamate and ϵ-*N*-benzyloxycarbonyl-L-lysine in 1% solution of absolute benzene at room temperature using sodium methoxide as the initiator ($[M_0]/[I_0]$ = 200); the benzyl and benzyloxycarbonyl groups were subsequently removed with dry hydrogen bromide. The resulting copolypeptides had an average molecular weight ranging from 36 000 to 110 000,

TABLE 16. Dependence of the composition of copolypeptides on the composition of the initial mixture.

Molar composition of initial mixture, %				Molar composition of copolypeptides, %			
L-glutamic acid	L-lysine	L-phenyl-alanine	L-tyrosine	L-glutamic acid	L-lysine	L-phenyl-alanine	L-tyrosine
50.0	33.3	—	16.7	49.8	34.3	—	15.9
47.6	38.4	—	4.0	56.4	37.8	—	5.8
58.0	42.0	—	—	59.0	41.0	—	—
58.0	41.0	—	1.0	56.1	42.6	—	1.3
58.0	38.0	4.0	—	61.6	33.4	5.0	—
56.0	36.0	8.0	—	57.1	33.9	9.0	—
—	100.0	—	—	—	100	—	—
100	—	—	—	100	—	—	—

and were readily soluble in water both at low and high pH, the solubility being a minimum in the region of neutral pH and depending on the composition and charge of the polypeptide, i.e. as regards their solubility they resembled polyamphoteric compounds and certain proteins.

In order to elucidate the dependence of the conformation of macromolecules on the composition of copolymers, the copolypeptides of *S*-methyl-L-cysteine and L-methionine with molar ratios of 1 : 9, 2 : 8, 3 : 7, and 5 : 5 and the copolymers of L-valine and L-methionine with the same molar ratios were synthesised[324] from the anhydrides of the corresponding *N*-carboxy-derivatives in nitrobenzene solution (4%) using sodium methoxide as the initiator ($[M_0]/[I_0]$ = = 200). For the same purpose, Karlson et al.[144] synthesised poly-β-benzyl esters of L- and D-aspartic acid, the poly-γ-benzyl ester of glutamic acid, and also a series of their copolymers by the polymerisation of a mixture of the anhydrides of the *N*-carboxy-derivatives in methylene chloride solution using sodium methoxide as the initiator ($[M_0]/[I_0]$ = 200) with different monomer ratios (1 : 9, 2 : 8, 3 : 7, 5 : 5, 7 : 3, 9 : 1). It was established that poly(β-benzyl L-aspartate) and poly(γ-benzyl L-glutamate) have an α-spiral conformation but with the opposite direction of turns.

To study the effect of the ester group on the conformation and properties of poly-β-aryl esters of L-aspartic acid, a number of copolymers with different molar ratios of ester residues were prepared[302] by the copolymerisation of the anhydrides of β-*p*-nitrobenzyl and β-benzyl esters of *N*-carboxy-L-aspartic acid in dimethylformamide using diethylamine as the initiator. It was found that the copolymers have a spiral conformation, the introduction of more than 35–40% *p*-nitrobenzyl residues into poly(β-benzyl L-aspartate) reversing the direction of the turns of the spiral.

By the copolymerisation of the anhydride of γ-benzyl *N*-carboxy-L-glutamate with the anhydrides of the *N*-carboxy-derivatives of L-alanine, L-leucine, and L-methionine at different ratios, Kulkarni and Blout obtained copolypeptides of interesting structure[325]. The γ-ester groups in the copolymers were replaced by 2-morpholinoethylamine residues. Such copolypeptides were readily soluble in water and showed a high content of the spiral conformation in solution. Addition to the solution of denaturing agents (urea) and salts caused their transition to a randomly coiled conformation.

To elucidate the influence of the steric configuration of the monomers and their ratio on the molecular weight and properties of the resulting copolypeptides[326], a number of polypeptides were synthesised by the copolymerisation of a 3% solution of a mixture of the anhydrides of the *N*-carboxy-derivatives in benzene saturated with water (70°, 24 h). The anhydrides of the *N*-carboxy-derivatives of the strongly basic D- or L-lysine and the acidic L-tyrosine were taken in 1 : 1 and 3 : 1 ratios. For the 3 : 1 monomer ratio, the resulting copolymers had a mean molecular weight of about 18 000 regardless of the configuration of the lysine. When the monomer ratio was 1 : 1, copolymers with the molecular weight $\overline{M}_W$ = 18 000 and $\overline{M}_W$ = 40 000 were obtained in the case of DL-lysine and D-lysine respectively as well as two polymers with different molecular weights in the case of L-lysine. The product with the higher molecular weight ($\overline{M}_W$ = 600 000) had a much higher content of L-tyrosine than the copolymer with the lower molecular weight ($\overline{M}_W$ = = 13 000).

To synthesise polypeptides having a structure similar to that of proteins, polypeptides were prepared[327] by the copolymerisation (using 0.5 mole % of sodium methoxide) of an equimolar mixture of the anhydrides of the *N*-carboxy-derivatives (4% solution in dimethylformamide) of L-lysine and L-glutamic acid, whose homopolymers have the α-spiral conformation at pH above 10.5 and below 4.5 respectively. It was expected that such copolymers would have the α-spiral conformation at a neutral pH. The resulting copolymers had a molecular weight of about 40 000 and were soluble in water. Up to 15% of the spiral conformation was detected in a neutral 0.15 M sodium chloride solution, the amount increasing to 50% at pH 4 and falling to zero at pH 12 or on removal of sodium chloride.

In an attempt to synthesise soluble amphoteric polypeptides, a study was made of the copolymerisation of the anhydrides of the *N*-carboxy-derivatives of L-glutamic acid, L-lysine, and L-phenylalanine in the ratios 1 : 1 : 3 and of L-glutamic acid, L-lysine, and L-leucine in the ratios 1 : 1 : 1.9.[328–330] The resulting polypeptides had typical amphoteric properties. They dissolved in dilute mineral acids and alkalis but not in water at pH between 4.0 and 10.8, which the authors of these studies explained by the aggregation of the copolypeptides on approach to the isoelectric point, which for the second polymer is 7.3.

Later Yuki[329] found that, in order to increase the solubility in the region of neutral pH, it is necessary to introduce serine residues, which together with threonine residues are responsible for the solubility of albumin. Copolypeptides containing L-glutamic acid and DL-serine in molar ratios of 4 : 1, 3 : 2, 2 : 3, and 1 : 4 were obtained. The last two copolymers were soluble even in water. The free copolymer containing L-glutamic acid, L-lysine, and DL-serine in the proportions 1 : 1 : 2 dissolved at any pH. The specific viscosity of this copolymer was found to be related to the pH (Fig. 19). The viscosity is a minimum at the isoelectric point (pH 7), which is characteristic of amphoteric polyelectrolytes.

Linear water-soluble copolypeptides of L-glutamic acid, L-lysine, and L-tyrosine in the proportions 2.8 : 2.6 : 1.0 have also been obtained[331], by the polymerisation of the anhydrides of the corresponding *N*-carboxy-derivatives in benzene solution using sodium methoxide as the initiator ($[M_0]/[I_0]$ = 400), with subsequent removal of the benzyloxycarbonyl group. The copolymer had $\overline{M}_W \simeq$ 49 000 and was soluble in water, but it dissolved in 0.15 M sodium chloride solution only at pH $>$ 10.

The following tyrosine-containing copolymers have been prepared[332] from the anhydrides of the corresponding *N*-carboxy-derivatives: glycine–L-tyrosine with molar ratios 1 : 1 ($\overline{M}_W$ = 7390) and 2 : 1 ($\overline{M}_W$ = 6520); L-phenylalanine–L-tyrosine with molar ratios 1 : 1 ($\overline{M}_W$ = 6370) and 2 : 1 ($\overline{M}_W$ = 8780); and glycine–DL-alanine–L-tyrosine with molar ratios 8 : 4 : 1 ($\overline{M}_W$ = 2360).

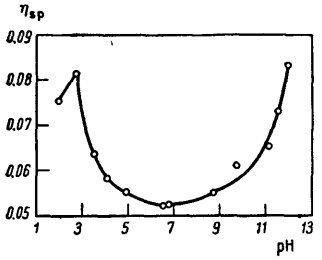


Fig. 19. Dependence of the intrinsic viscosity of the 1 : 1 : 2 glutamic acid–L-lysine–DL-serine copolymer on the pH of the medium.

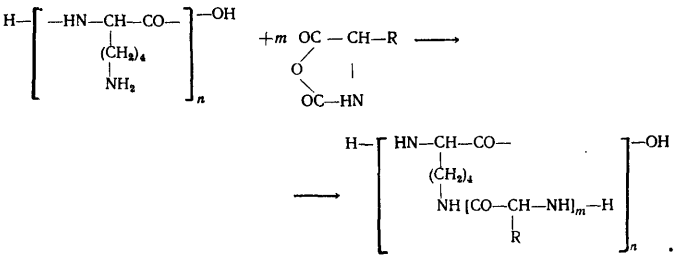
Since many physical and chemical properties of collagen and gelatine are determined by their large content of proline and hydroxyproline, model polypeptides containing the above amino-acid residues were synthesised[333]. Copolymers with the following monomer ratios were obtained by the copolymerisation at room temperature of a mixture of the anhydrides of the corresponding *N*-carboxy-derivatives in dioxan solution with diethylamine and triethylamine as the initiators or in pyridine without initiator: L-proline : glycine = 1 : 1, 1 : 2, and 1 : 3; L-proline : glycine : L-hydroxyproline = 1 : 2 : 1.

Copolypeptides containing L-arginine and L-leucine (2.3 : 1), which have a weak inhibiting effect on blood coagulation[334], and L-glutamic acid, 2,5-dihydroxyphenylalanine, and 3,4-dihydroxyphenylalanine, with weak catalytic properties[335], have been described.

Patents dealing with the method of preparation of copolypeptides and their application in various branches of industries have appeared in recent years. Thus a patent describes the preparation of copolypeptides by the treatment of solutions of the sodium salts of amino-acids (obtained from proteins, for example serum albumin) with the esters of chloro-acids, for example methyl chloroformate, and then with thionyl chloride, which yields a mixture of the anhydrides of the *N*-carboxy-derivatives; the latter is polymerised by the usual methods and converted into fibres and films[336]. Another patent[337] describes the method of preparation of copolypeptides from a mixture of the anhydrides of the *N*-carboxy-derivatives of L-leucine and phenylalanine. The molecular weight of the resulting copolypeptides exceeds 7000. These copolymers can be used for the preparation of fibres, sheets of various types, and moulds.

5. Preparation of Graft and Block Copolymers

Proteins react under mild conditions with the anhydrides of *N*-carboxy-derivatives of α-amino-acids with the formation of polypeptidyl proteins. Like synthetic polypeptides with free amino-groups, proteins with free amino-groups function as polyinitiators in these reactions. Branched polyamino-acids have been prepared[338] by the polymerisation of the anhydrides of the *N*-carboxy-derivatives of DL-alanine, sarcosine, and L-lysine in the presence of poly-L-lysine, which functioned as the polymerisation initiator. Poly-(ϵ-*N*-poly-DL-alanyl)-L-lysine with n = 20 and m = 20, water-soluble poly(ϵ-*N*-polysarcosyl)-L-lysine with n = 20 and m = 7, and branched poly(ϵ-*N*-poly-L-lysyl)-L-lysine with n = 30 and m = 15 have been obtained.



Similarly an aqueous solution of the hydrobromide of poly-L-lysine was used as a polyvalent initiator[339] for the polymerisation of the anhydride of γ-benzyl *N*-carboxyglutamate; the product was a branched polypeptide with n = 280 and m = 40, whose molecular weight was 1 500 000.

Poroshin[340] studied the reaction of the products of the primary degradation of casein (peptone hydrolysis mixture) with the anhydrides of the *N*-carboxy-derivatives of glycine, DL-alanine, and DL-proline in cooled dioxan solutions or in phosphate buffer. The reaction products were dialysed and then examined for their amino-acid content. It was established that in the case of polyglycylpeptone the degree of polymerisation (m) of glycine was 8–10 while for poly-DL-alanylpeptone m was 18–20. The poly-DL-alanylpeptone was used for the polymerisation of the anhydride of *N*-carboxy-DL-proline, the products being poly-DL-alanyl--poly-DL-prolylpeptone which was readily soluble in water.

Konigsberg and Becker[341] grafted the anhydrides of the *N*-carboxy-derivatives of glycine and phenylalanine, with a [^{13}C]carboxyl carbon atom, on to a number of proteins (albumin, egg albumin, chymotrypsin, chymotrypsinogen, and lysosyme) in 0.067 M phosphate buffer at pH 6.2–7.8, in 0.1 M borate buffer at pH 8.2–9.1, and in sodium bicarbonate solution saturated with carbon dioxide, at pH 6.9–7.6. A gradual increase in the amount of grafted glycine was observed when the pH was altered from 6.2 to 9. Among the above solutions, the bicarbonate solution proved to be the most effective. For example, in phosphate buffer 41 moles of glycine residues were grafted on to one mole of protein, while under otherwise the same conditions in sodium bicarbonate solution 72 moles of glycine residues were grafted on to one mole of protein.

Kley et al.[342,343] treated bovine and rabbit blood albumin in bicarbonate solution or in phosphate buffer with the anhydrides of the *N*-carboxy-derivatives of glycine, leucine, phenylalanine, glutamic acid, and lysine. Microtitration showed an appreciable change in the amounts of carboxy-, imidazole, and α- and ϵ-amino-groups and established that grafting takes place partly (to the extent of 30%) at ϵ-amino-groups.

A water soluble gliadine fraction was obtained by peptidylation[344]. For this purpose, DL-alanine and N^4-(3-hydroxypropyl)-L-asparagine were grafted on to gliadine. In the first case the reaction was carried out with the anhydride of *N*-carboxy-DL-alanine and gliadine in a mixture of dioxan and 0.05 M phosphate buffer (pH 6.8, 2°, 48 h), and water soluble poly-DL-alanylgliadine was isolated. The alanine content increased from 1.4% in gliadine to 23.6% in poly-DL-alanylgliadine, which corresponds to the grafting, on average, of 4.1 millimoles of alanine residues per gramme of gliadine. In the second case the reaction was

carried out with the anhydride of benzyl *N*-carboxyaspartate and gliadine in dimethylformamide (20°, 48 h). After its isolation, the polybenzyloxy-L-aspartylgliadine was kept for 30 min at 40° in propanolamine; the water-soluble *N*-(3-hydroxypropyl)-L-aspartylgliadine, enriched in aspartic acid residues up to 6.3 millimoles per gramme of gliadine, was then isolated by precipitation with 4 M acetic acid.

As pointed out by Katchalski and Sela[5], peptidylation does not interfere in most cases with the biological properties of proteins, but modifies a number of their other properties. Bearing in mind the stability of valine peptides towards acid hydrolysis, a study was made of the effect of grafting valine on to proteins on the biological and chemical properties of the latter[345]. Poly-DL-valylchymotrypsin, poly-DL-valyllysosyme, and poly-DL-valylinsulin were obtained by the reaction of the anhydride of *N*-carboxy-DL-valine with proteins at 4° in phosphate (pH 7.6) and borate (pH 8.5) buffers. The modified proteins are unusually stable towards acid hydrolysis. Hydrolysis is complete only after boiling with hydrochloric acid for 96 h. The enzymic activity of poly-DL-valylchymotrypsin is reduced only by 60% compared with the activity of the natural protein, while in the case of polyglycylchymotrypsin the activity is the same as that of the natural enzyme.

The effect of grafting L-tyrosine, L-tryptophan, L-phenylalanine, L-cysteine, L-proline, L-glutamic acid, L-lysine, L-alanine, L-serine, L-cyclohexylalanine, a mixture of L-glutamic acid and L-tyrosine, and of L-glutamic acid and L-cyclohexylalanine on the physiological activity of gelatine has been investigated[346]. It was found that when amino-acids with aromatic and cyclohexane rings in the side-chains are grafted on to gelatine, the physiological activity of the latter increases.

The problem of converting certain enzymes into water-insoluble products, with specific catalytic activity, is interesting because the "water-insoluble enzymes" can be readily removed from the reaction sphere. They can also be used for the preparation of columns with enzymic activity. For this purpose, Atara Bar-Eli and Katchalski[347] prepared polytyrosyltrypsin which was completely soluble and retained the enzymic activity of trypsin. When it was combined by diazotisation with the copolymer of *p*-aminophenylalanine and leucine, trypsin which was completely insoluble in water but nevertheless retained its enzymic activity was obtained. The latter could be used for the preparation of an active trypsin column for systematic amino-acid analysis.

Polytyrosyltrypsin containing 20–28 tyrosine residues grafted per mole of trypsin was prepared[348] by the polymerisation of the anhydride of *N*-carboxy-L-tyrosine in aqueous solution using trypsin as a polyvalent initiator. It was established that approximately half of the ϵ-amino-groups of trypsin were acylated by the anhydride of *N*-carboxy-L-tyrosine, the average chain length of the grafted tyrosine being about 2.5. In contrast to trypsin, polytyrosyltrypsin is moderately soluble at pH 5–9. In its response to denaturing agents (urea, soya bean oil) and in its enzymic activity polytyrosyltrypsin is indistinguishable from trypsin.

A water-soluble block polypeptide consisting of a central poly-L-alanine block and two side poly-DL-glutamic acid blocks was synthesised[349]. The synthesis was carried out in benzene solution, the initial concentration of the anhydride of γ-benzyl *N*-carboxy-DL-glutamate being 2%. The initiator was n-hexylamine to which the anhydrides of *N*-carboxy-L-alanine and then of γ-benzyl *N*-carboxy-DL-glutamate were added in succession. The course of polymerisation was followed from the carbon dioxide evolved. According to the amino-acid analysis, the molar ratio of glutamic acid to alanine was 1.54. It was established that the molecule of the block polymer had a block consisting of 175 residues of L-alanine and two DL-glutamic acid blocks consisting of 325 residues altogether.

Any polymeric material containing free primary or secondary amino-groups can be used as a polyvalent initiator of the polymerisation of the anhydrides of *N*-carboxy-derivatives and not only natural proteins or synthetic poly-α-amino-acids. Thus polymerisation of styrene in liquid ammonia with sodium as the initiator at -40°, with subsequent addition of ammonium chloride and removal of ammonium by distillation, yielded polystyrene containing 11% of nitrogen. The polymerisation of the anhydride of *N*-carboxyl-DL-valine in benzene solution of this polystyrene gave a product containing 24–29% of polystyrene and soluble only in *m*-cresol. The copolymers styrene–glycidyl methacrylate–DL-phenylalanine and styrene–glycidyl acrylate–DL-phenylalanine were prepared similarly[350]. These unusual polyamides are thermoplastic and exhibit exceptional stability towards solvents.

1. E. Fischer, Ber., 34, 2868 (1901); 37, 2504 (1904); 38 605, 2914 (1905); 39, 471, 2893 (1906); 40, 1754 (1907).
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COPOLYMERISATION OF HYDROCARBONS IN THE PRESENCE OF IONIC CATALYSTS

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1. INTRODUCTION

Comparison of the relative activities of monomers in copolymerisation has been successfully used to study the mechanism of chain growth in radical polymerisation. The copolymerisation constants yield quantitative estimates of the relative reactivities of monomers and of the corresponding active centres.

During recent years a great deal of attention has been paid to catalysts which bring about polymerisation by an ionic mechanism, in connection with the use of catalysts of ionic type for the synthesis of several polymers having valuable properties, especially stereoregular polymers. Data on copolymerisation provide a deeper insight into the mechanism of chain formation in cationic, anionic, and coordination–ionic polymerisation.

2. THEORETICAL CONCEPTS

A theoretical treatment of the problems involved in radical copolymerisation has been given, and we must therefore examine the possibility of applying to ionic polymerisation the relations obtained. Scheme (1), suggested by Alfrey and Goldfinger[1], considers four possible modes of chain growth in the copolymerisation of two monomers: i.e. the reactivity of the growing polymeric ion is assumed to depend on the nature of the terminal active unit in the chain, and the rate constant for chain growth to be determined by the reactivity both of the monomer and of the active centre. Here M* denotes a cation in the case of cationic polymerisation, and an anion in anionic polymerisation, while in the case of coordination-complex polymerisation it represents a catalytic complex bound to the active end of the growing chain.

$$\begin{aligned} &\sim M_1^* + M_1 \xrightarrow{k_{11}} \sim M_1^*, \\ &\sim M_1^* + M_2 \xrightarrow{k_{12}} \sim M_2^*, \\ &\sim M_2^* + M_2 \xrightarrow{k_{22}} \sim M_2^*, \\ &\sim M_2^* + M_1 \xrightarrow{k_{21}} \sim M_1^*. \end{aligned} \qquad (1)$$

When the concentration of growing chains is constant, we can apply the relationship that has been termed the fundamental equation of copolymerisation[1,2]

$$\frac{d[M_1]}{d[M_2]} = \frac{[M_1]}{[M_2]}\left(\frac{r_1[M_1]+[M_2]}{r_2[M_2]+[M_1]}\right), \qquad (2)$$

where $r_1 = k_{11}/k_{12}$ and $r_2 = k_{22}/k_{21}$ are copolymerisation constants for the first and second monomers, representing the relative activities of the monomers towards addition to the active centres $\sim M_1^*$ (r_1) and M_2^* (r_2). Eqn. (2) is applicable to ionic copolymerisation only if the concentration of active centres remains constant during such processes. The necessity for this condition had already been noted by Landler[3,4], who was the first to generalise data on ionic polymerisation.