1964

THE STRUCTURAL RELATIONSHIPS OF SOME GLYCINE POLYMERS

WILLIAMS, ROY ALAN

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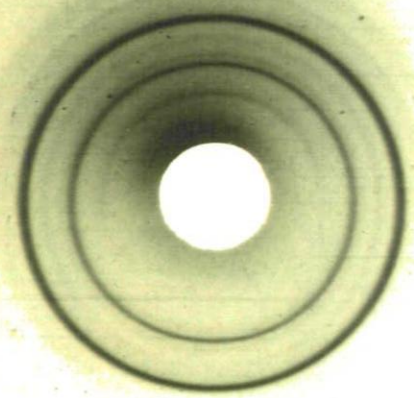
A Thesis submitted for the degree of Doctor of Philosophy by

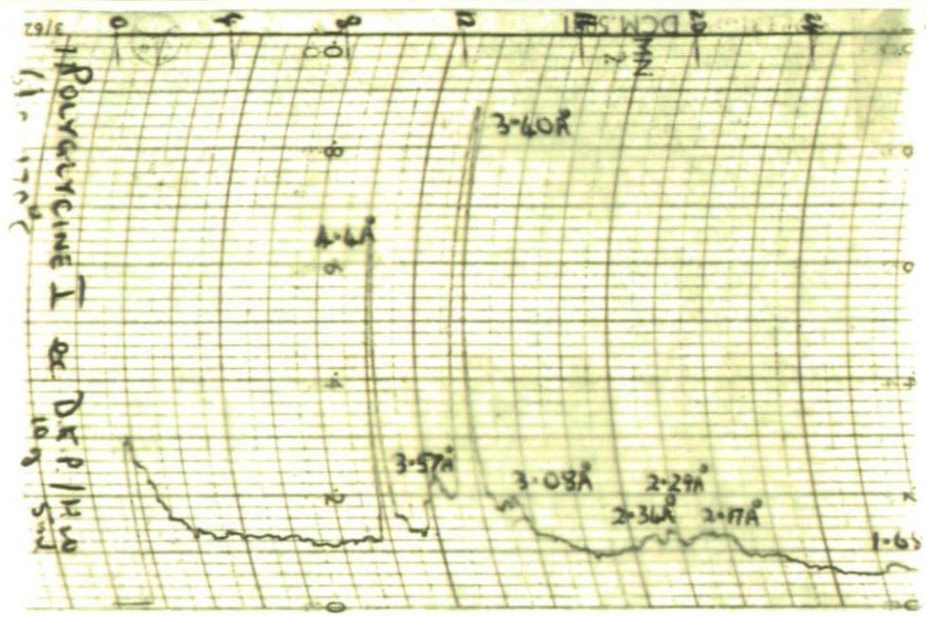
ROY ALAN WILLIAMS.

Department of Chemistry,
Plymouth College of Technology,
PLYMOUTH.

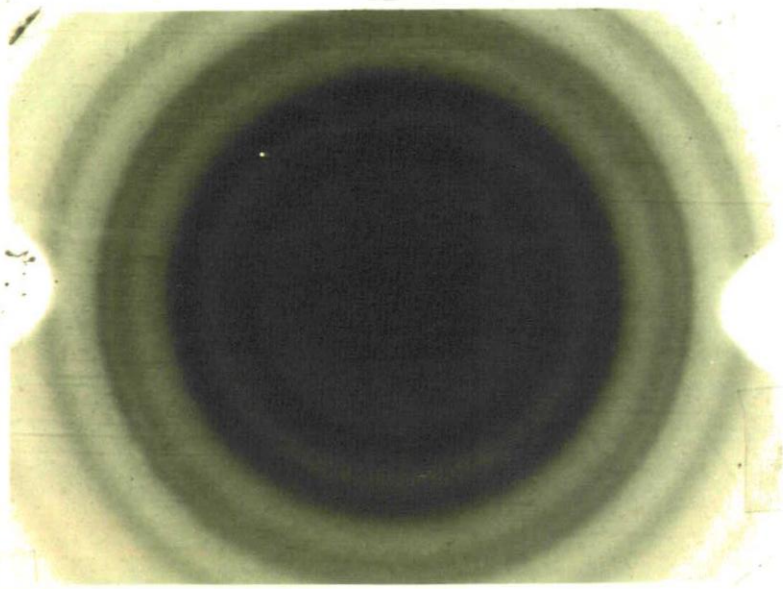
May 1964.

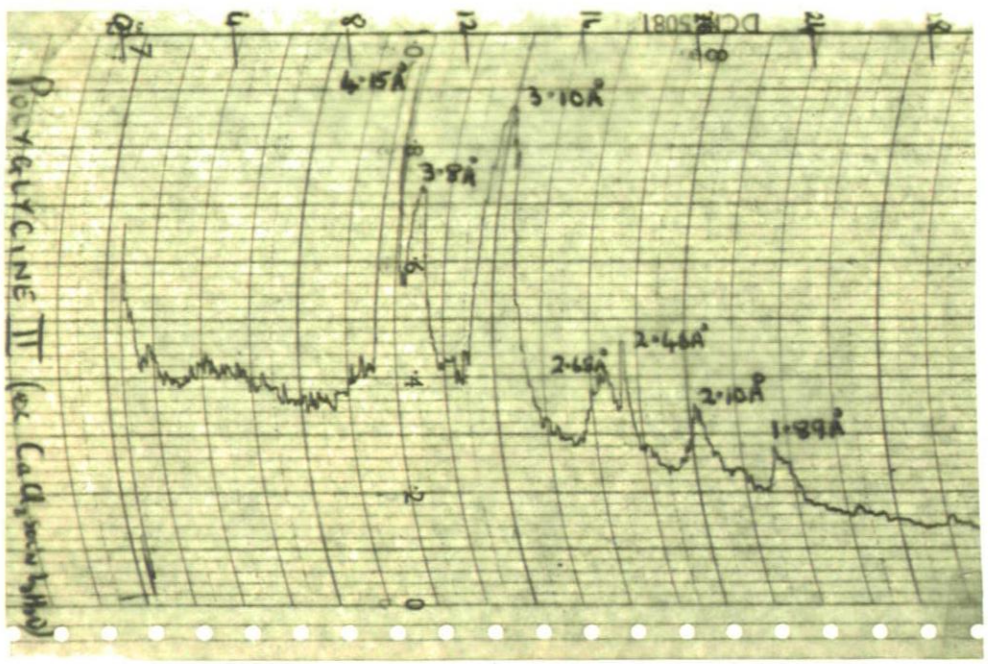
POLYGLYCINE I.
(ex. DIKETOPIPERAZINE AND WATER)





POLYGLYCINE II.
(ex. POLYGLYCINE I SOLUTION IN CALCIUM CHLORIDE + WATER)





SERIOUS ERRORS

Summary. P. 49. Re. Crick & Rich. Diagram.
Should read THEIR FIGURE.

Exp 15. p. 14 a: 2·63Å spacing should be 2·53Å.

Exp. 16. p. 4. Peak 10° 38 should be peak 10° 8'.

Exp. 16. p 8. Chart 14 should read. Chart 13.

References to D.K.P. structure.

1). K. Lonsdale. Acta Cryst. (1961), 14, 37.
lines (X-rays).
2). Degeilh R. & Marsh R.E. (1959)
Acta Cryst. 12, 1007.

INDEX TO THE X-RAY DIFFRACTOMETER CHARTS.

The chart traces are indexed by numbering, from left to right across the sheet, and from the top to the bottom of the sheet.

CHART No. 1.

1) The product from the melt of glycine(10 g.) and benzoyl glycine(10.5 g.), heated in the open. The product crystallised from a boiling water extract of reaction mixture, on filtration and cooling.
2) This sample was deposited from the cold filtrate obtained after the removal of sample 1) above, and allowing the filtrate to stand overnight.
3) The bulk residue from the polymerisation in the melt of glycine(10 g.) and benzoyl glycine(10.5 g.).1 st. prep'n.
4) A sample of the benzoyl glycine used above.
5) The bulk residue from the second preparation of the glycine and benzoyl glycine polymerisation product.

CHART No. 2.

1) Product from the polymerisation of glycine(5 g.) and phenol/water(5 ml.)[the upper phase], heated in a sealed tube at 140°C., for four weeks.
2) Product from heating glycine(5 g.) alone in a sealed tube, for one week at 180°C.

CHART No. 2 (continued).

3) The product from the polymerisation of glycine(10 g.), when heated in diphenylamine, in a sealed tube for 2 weeks, at 150°C.

4) Glycine(5 g.) was heated with water(1 ml.) in a sealed tube at 160°C., for 48 hours. The insoluble residue was washed with water and alcohol, and centrifuged down and dried. This sample was taken from the bottom of the centrifuge tube.

5) Polyglycine I prepared by the polymerisation of diketopiperazine(10 g.) in water(5 ml.), by heating in a sealed tube for 6 hours at 170°C.

6) See trace 4) above. This sample was taken from the top of the centrifuge tube.

CHART No. 3.

1) The polymerisation of glycine(5 g.) in hydrochloric acid(3 ml. conc.), heated sealed at 140°C. for 7 hours. This sample was taken from the top portion of the carius tube, which had been totally liquid at 140°C..

2) See trace 1) above. This sample was taken from the bottom of the carius tube, which had contained solid at 140°C.

3) The same reaction mixture as above was heated for 11 hours. This sample was taken from the upper liquid, at 140 C., portion of the carius tube.

CHART No. 3 (continued)

4) See trace 3) above, the sample was taken from the lower portion of the carius tube, which contained solid at 140°C.

5) The same reaction mixture was heated for 14 hours. This sample was taken from the upper portion of the cooled reaction mixture.

6) See trace 5) above, this sample was taken from the lower portion of the reaction mixture.

7) The same reaction mixture was heated for 24 hours at 140°C.. This sample was taken from the washed solid extracted from the total reaction mixture.

8) The same reaction mixture was heated at 200°C. for 24 hours. This sample was the bulk low molecular weight peptides of glycine, which were soluble in hot water, but could be precipitated from the cold solution by adding ethanol.

CHART No. 4.

1) The bulk washed product from the polymerisation of glycine (40 g.) in concentrated hydrochloric acid(16 ml.) by heating in a sealed tube at 150°C. for 24 hours.

2) Polyglycine II, prepared by repeated reprecipitation from solutions in saturated aqueous calcium chloride solution, by the addition of water.

CHART No. 4(continued).

3) Polyglycine II(donuts), prepared by repeated precipitation from solution in 70% aqueous zinc chloride solution, on the addition of water.

4) Polyglycine II(thick hexagonal microcrystals), from the repeated reprecipitation from solution in saturated aqueous calcium chloride solution, by water.

5) Polyglycine, prepared by the precipitation of a 5% solution of Polyglycine I in 70% aqueous zinc chloride solution(5 ml.) by 60 ml. of industrial spirit, and washed with industrial spirit.

6) Polyglycine prepared by precipitating 5 ml. of 10% solution of Polyglycine I in 70% aqueous zinc chloride solution, by 60 ml. of industrial spirit.

7) Polyglycine, from the polymerisation of glycine(5g.) in concentrated hydrochloric acid(1ml.) in a sealed tube at 155°C. for 84 hours. The sample was taken from the top crust of the centrifuged water insoluble residue.

CHART No. 5.

1) Diketopiperazine(5 g.) and water(5 ml.) heated sealed at 160°C. for 4 hours. The sample was taken from the water and ethanol washed, water insoluble residue.

2) Diketopiperazine, prepared from glycine and glycol.

CHART No5. (continued)

3) Diketopiperazine and water(5 g. and 2½ ml.) heated sealed at 160°C. for 5 hours. The sample is taken from the alcohol and water washed residue.

4) Diketopiperazine(10 g.) and water(7 ml.) heated sealed for 6 hours at 160°C. The sample is taken from the washed insoluble residue.

5) Polyglycine, prepared by boiling carbenzoxy-glycine anhydride in pyridine.

6) Polyglycine, prepared from carbenzoxy-glycine anhydride polymerisation in the presence of water vapour, at room temperature.

CHART No. 6.

1) Analar glycine.(B.D.H.)

2) Glycine Hydrochloride, prepared by the Frost method.

3) Glycine Ethyl Ester Hydrochloride (Mpt. 145°C.).

4) Diglycine Hydrochloride, Frost method(Mpt. 186°C.).

5) Diketopiperazine, prepared from glycine and glycol.

6) Glycylglycine Hydrochloride Hydrate, (Mpt. 141°C.).

CHART No. 7.

1) Glycylglycine Hydrochloride hydrate (Mpt. 141°C.)

2) Glycylglycine Ethyl Ester Hydrochloride, prepared by the Schott, Larkin, Rockland and Dunn method, (Mpt. 185-186 °C.)

CHART No. 7.(continued)

3) Glycylglycine Methyl Ester Hydrochloride,(Mpt. 189°C.)

4) Diglycylglycine, recrystallised from ethanol/water, [Mpt. 246°C.(d.)].

5) Diglycylglycine Ethyl Ester Hydrochloride,(Mpt. 216°C.)

6) Diglycylglycine Methyl Ester Hydrochloride, (Mpt. 196°C).

CHART No. 8.

1) Triglycylglycine , [Mpt. 280 - 270°C(d)].

2) Pentaglycylglycine.

3) Pentaglycylglycine methyl ester hydrochloride.

4) Polyglycine II, repeated reprecipitation from solution in calcium chloride.

5) Polyglycine I, ex. diketopiperazine(10g.) heated in water(5 ml.), in a sealed tube at 170°C. for 6 hours.

CHART No. 9.

1) Polymerisation of glycine(60 g.) in concentrated phosphoric acid(16 ml.) heated in the open at 164°C. for 10 minutes. The sample was taken from the hot water washed insoluble residue.

2) See trace 1) above, the sample was taken from the solid which crystallised on cooling out of the first hot water extraction of the reaction mixture.

4) See trace 2) above, deposit from the filtered solution on standing overnight.

CHART No. 9 (continued).

4) A sample taken from the second hot water soluble fraction

5) See trace 4), solid extracted from the first hot water extraction solution, on adding excess ethanol.

6) See trace 5), solid extracted from the second hot water extraction solution, on adding alcohol.

CHART No. 10.

1) Polymerisation of glycine(20 g.) and concentrated phosphoric acid(20 ml.) heated in the open for 30 minutes at 170°C. A sample of the black water insoluble product.

2) See trace 1), leaflets deposited on cooling from the hot water extraction.

3) Hot water washed polymer from the polymerisation of glycine (40 g.) in phosphoric acid(8ml.), dried in air. The sample was taken from the grey bottom residue of the centrifuged product.

4) See Trace 3), sample taken from the dark top crust of the dried centrifuged product.

5) See trace 3), a ground unorganted sample of the hot water soluble portion of the product.

6) See trace 3), low molecular weight peptide from the second hot water extraction of the reactionmixture.

7) See trace 6), deposited on adding ethanol to the solution from the second extraction.

CHART No. 11.

1) Polymerisation of glycine(30 g.) in phosphoric acid (15 ml.) by heating in the open at 164°C. Sample from the residue on cooling the third hot water extract.

2) See trace 1) above, deposit obtained on treating the filtrate from above with excess ethanol.

3) See trace 1) above, solid deposited on cooling from the fourth hot water soluble extract, the sample was used as oriented by centrifuging down.

4) Polymerisation of glycine(20 g.) in phosphoric acid (5 ml.), by heating in a sealed tube at 170°C., for 21 hours. The sample is taken from the first recrystallisation of the hot water soluble fraction. This sample showed hexagonal leaflets under the electron microscope.

5) Polymerisation of glycine(30 g.) in phosphoric acid (5 ml.), by heating sealed at 157°C. for 24 hours. This sample was taken from the hot water soluble polymer.

6) See trace 5) above. This sample was taken from the bulk water washed polymer.

7) Polymerisation of glycine(5 g.) in phosphoric acid(1 ml.), by heating sealed at 147°C. for 24 hours. The reaction mixture was extracted with hot water, the polymer precipitated on cooling filtered off, and the solution allowed to stand, this sample was then deposited.

CHART No. 12.

1) This sample was thought to be glycylglycine ethyl ester hydrochloride, but was shown by the single crystal analysis to be totally converted to diglycine hydrochloride.
2) Hot water soluble Polyglycine II, obtained from the preparation of diketopiperazine, by the dimerisation of glycine in glycol.
3) Diglycine hydrochloride, prepared by the Frost method.
4) The sample was taken from the dried brown sludge obtained during the preparation of diketopiperazine, from glycine and glycol.
5) Glycylglycine ethyl ester hydrochloride, prepared by the method of Schott, Larkin, Rockland and Dunn.
6) Glycine hydrochloride, prepared by the method of Frost.

CHART No. 13.

This chart was one of several traces made for samples of Polyglycine II, reprecipitated from solutions in calcium chloride solution by water. The chart speed was 30 inches per hour. The numbers on the chart refer to this chart only. The numbers in the circle refer to the numbered peaks given in the list on page 8a(Exp. 16). The near horisontal line is an attempt to drew a mean background level.

ACKNOWLEDGEMENTS.

I wish to thank.-

The Governors of the Plymouth College of Technology for the award of a Research Studentship.

My supervisor Dr. A. B. Meggy for his help and encouragement.

All my friends at the College of Technology for their helpful suggestions and valuable discussion.

And my wife Sylvia, for her forbearance, and help in the preparation of this work.

THE INDEX.

THE INTRODUCTION.

INTRODUCTION.

The Polyglycines are of interest because they are related to nylon and the polyamides, and to the proteins. They resemble nylon in having amine, $-NH_2$, end groups, and the chains are formed through peptide, $-CO-NH-$, linkages. The known Polyglycines are not fibre forming, as products with the required degree of polymerisation have not been prepared; they have a low degree of polymerisation, about 10 - 20 is the usually recorded value for the number average degree of polymerisation. The degree of polymerisation of the fibre forming polyamides is about 200. The polyglycines may be regarded as the simplest proteins, in that they only contain one amino-acid species, and this is the simplest one.

Peptides of glycine have been known for many years. The earliest recorded preparation of a glycine polymer was that of Curtius[41](1888). The earliest recorded preparation of Polyglycine was by Maillard[86](1914). He obtained a water insoluble horny polymer. The discovery that Polyglycine existed in two different forms, two different crystalline modifications, was made by Meyer and

Go[92](1934). They showed that the two forms had different X-ray diffraction patterns. This work was revived by Elliott and Malcolm[48](1956), who showed that the two Polyglycines had different Infrared Spectra. The polymers were named Polyglycine I and Polyglycine II. Polyglycine I was believed to be related to the structure shown by silk. Pauling and Corey[95](1951) postulated a "pleated sheet" structure, in which the chains were extended, and the structure showed a two-fold screw axis. Polyglycine II did not have a structure which could be related to any other known peptide, or protein structure. A structure has been postulated by Crick and Rich[39](1955), in which the chains were hexagonally packed, and extended, the structure showed a three-fold screw axis. The structural relationships, and the relative stabilities of the two forms is not known. No other crystalline modifications of Polyglycine have been discovered; in particular no alpha helix structure has been observed. The alpha helix structure is common among the proteins.

The purpose of this work was three-fold.-
1) to see if any new forms of Polyglycine could be found.

2) to determine whether the transition between Polyglycine I and Polyglycine II was reversible.
3) if possible to obtain information leading to a better foundation for the suggested structure for the two crystalline forms of Polyglycine.

Many methods of preparation of both forms of Polyglycine have been examined. A new low molecular weight form of Polyglycine II has been discovered. This unlike Polyglycine II is soluble in hot water. The study of the polymerisation of glycine in phosphoric acid solution may also yield a high molecular weight form of Polyglycine I. The investigation of the various polymerisations has also produced evidence of the relative stabilities of the two forms of Polyglycine. In all the cases of the direct polymerisation of glycine which have been examined, Polyglycine II was always obtained as the initial product. Prolonged reaction, then converted the Polyglycine II into Polyglycine I. Analysis by paper chromatography of the reaction products of the polymerisation of glycine in hydrochloric acid, showed that diketopiperazine was present in the reaction mixture. This could only have been produced by the cyclic polymerisation of two molecules of glycine.

It has not been possible to confirm the transition between the two Polyglycines observed by Meggy and Sikorski[91](1956). There did not appear to be a true transition between the two forms. The results strongly indicated that Polyglycine II is a metastable form at all temperatures, with respect to Polyglycine I.

The X-ray diffraction examination of the products of various polymerisations has shown that many preparations contained mixtures of both Polyglycine I and Polyglycine II. It was not found possible to prepare any single crystal specimens of either form of Polyglycine. A probable method for obtaining single crystals has been obtained for both forms, of low molecular weight water soluble peptides. The crystalline quality of the specimens has been examined under the electron microscope. The growth during repeated recrystallisations of Polyglycine II has also been studied. This growth has been shown to pass through several stages, showing different crystal shapes, during the recrystallisation. The best crystalline samples showed near hexagonal leaflets. The hot water soluble low molecular weight specimen also showed hexagonal leaflet shape. This supported the

structure proposed by Crick and Rich. The spacings obtained from the X-ray powder photographs agreed with the proposed structure also. The microcrystals were then subjected to X-ray powder diffractometry. The chart trace showed that although agreement was good for all the spacings of the proposed structure, there were several additional spacings present which could not be accounted for by the proposed structure. Attempts have been made to provide an alternative structure without any success. The structure seemed to be too complicated to be solved by powder diffractometry.

An X-ray examination of the lower peptides has also been carried out. The powder diffractometer trace for the pentaglycylglycine peptide, showed considerable similarities to that for Polyglycine II. The powder diffractometer trace of diglycylglycine peptide was not of the same structure as that observed by Bernal[26](1931), although his material was a hydrate. The material examined was not hydrated.

The samples of Polyglycine I which were prepared were of poor crystalline quality. The X-ray powder photographs and the diffractometer traces yielded little new information on the

possible structure. The photographs and traces showed only broad peaks and bands, but the 3.45 A. peak observed by Astbury at a Bragg angle of 12° 55', has been shown to occur consistently at 13° 6'(3.40 A.). The unit cell for the structure is certainly not the monoclinic cell proposed by Astbury[8,9].(1948). The hot water soluble Polyglycine I, of low molecular weight, would have yielded better crystals if there had been sufficient time for their preparation.

The benzoylpentaglycylglycine, obtained from the polymerisation of glycine and benzoylglycine, pentaglycylglycine, and the product from the polymerisation of glycine in phosphoric acid were unusual. The X-ray diffractometer traces of these materials showed low Bragg angle reflections. Such low angle reflections have not been observed for glycine peptides to the knowledge of the author.

A systematic X-ray study of the methyl and ethyl esters of the lower glycine peptides was unsuccessful. The preparation of single crystals using aqueous solvents, produced good crystals but the esters had been extensively hydrolysed.

SUMMARY OF PREVIOUS WORK

A SUMMARY OF PREVIOUS WORK

PREPARATION OF GLYCINE POLYMERS

A) FROM GLYCINE

The earliest attempt to polymerise glycine directly, was made by Kohler (1865)[74], who heated glycine in a stream of hydrochloric acid gas; he obtained a green fluorescent product which was mainly 2,5-diketopiperazine. When Abderhalden and Komm (1924)[4], heated glycine, alone, in a sealed tube at 160°C, they obtained glycine anhydride, 2,5-diketopiperazine, as the main product together with some polyglycine.

Glycine was also found to polymerise when heated in high boiling solvents. Herzog and Krahn (1924)[67], heated glycine and phenol in sealed tubes. The product of 2,5-diketopiperazine was obtained in good yield. Using glycerol, Balbiano and Trasciatti (1900)[12], also obtained 2,5-diketopiperazine, only a small amount, and a horny material which was insoluble in all the normal solvents and gave glycine on hydrolysis. This reaction was reinvestigated by Maillard (1911, 1914)[85, 86], who concluded that the yields of cyclic anhydride and polymer varied with the time of heating

and the conditions, whether heated sealed or in the open. He obtained the horny polymer and showed that if the glycine was first dissolved in water, diluted with glycerol and heated to 170°C, 2,5-diketopiperazine was obtained in 86% yield. The latter has since been disputed. The method generally used to prepare 2,5-diketopiperazine is one using glycol described by Sannie (1942)[103], and modified by Schott and co-workers (1947)[104]; the yield for this is 62%.

Curtius and Benrath (1904)[40], obtained 2,5-diketopiperazine and glycine peptides, mainly the pentapeptide, by heating glycine in a sealed tube at a high temperature. They also obtained a mixture of the N-benzoyl derivatives of the di-, tri-, tetra-, and hexapeptides of glycine, by heating the silver salt of glycine with benzoyl chloride, or by heating glycine with N-benzoyl glycine ester, or N-benzoylglycylglycine. The chief product was the N-benzoyl derivative of the hexapeptide. The degrees of polymerisation of these products was based on an elementary analysis, and was not to be wholly relied upon.

Meggy (1956)[89] studied the polymerisation of glycine in aqueous media. He found that polymerisation could be made to occur by heating with hydrochloric acid in a sealed tube at 140°C. The product was polyglycine I. The yield of

polymer depended on the ratio of glycine to hydrochloric acid. The optimum proportions were 5 gms. of glycine to 1 ml. of 10 N. acid. No polyglycine was formed at 130°C, and if the tubes which had been heated at 140°C were kept at 130°C for 24 hours, the yield of polymer was reduced. The essential conditions for the reaction appeared to be a temperature above 140°C and the presence of glycine as a solid phase. Hydrochloric acid was not essential to the reaction; phosphoric acid, ammonium chloride, and sodium hydroxide were effective catalysts, or the reaction could be carried out using water only. Hydrochloric acid gave the best yield and quality of polymer. The reaction could also be carried out at atmospheric pressure. Sufficient water was added to the mixture of glycine and hydrochloric acid to dissolve all the solid at the boiling point. The solution was refluxed for 2 hours, and then evaporated. No glycine separated, glycine did separate if the solution was evaporated before refluxing. The boiling point of the solution rose steadily as the water was removed, and the residue became increasingly viscous. The temperature was held at 150-160°C for a few hours. The product was then extracted with water. Polyglycine was obtained but the yield varied greatly. It appeared that during the refluxing some lower peptides were formed, and their presence interfered with the crystallisation of glycine,

so that a supersaturated solution was obtained, which could be heated to a temperature at which the glycine could polymerise

B) FROM PEPTIDES

The results obtained for the polymerisation of di-, and tri-peptides of glycine were confusing. Herzog and Krahn (1924)[67], found that glycylglycine was converted almost quantitatively into the corresponding anhydride on heating in a sealed tube with m-cresol at 105°C. They observed that polymerisation of the tripeptide under the same conditions was more complicated, without giving any more details. Abderhalden and Komm (1924)[4] investigated the polymerination of glycylglycine and diglycylglycine when heated in water in sealed tubes at 160°C. They found that both peptides gave substantial yield of diketopiperazine. Maillard (1914)[86] claimed that an aqueous extract obtained from heating glycine in glycerol contained diketopiperazine and triglycylglycine. On standing, this extract deposited the hexapeptide of glycine as a white solid. When this precipitation was complete the aqueous liquor no longer gave the biuret reaction, showing that the tetrapeptide had been used up. The polymerisation may have ceased at this stage, because of the insolubility of the hexapeptide. This observation suggested that diketopiperazine reacted readily with aqueous solutions of glycyl peptides at room temperature. It seemed that either diketopiperazine reacted only with the tetrapeptide, or that Adderhalden and Komm did not have favourable conditions for

water, and would not pass through a dialysing membrane. He did not record the nature of these products. Polyakova and Vereschagin (1949)[98] heated diketopiperazine in water at 170°C. at 200 to 400 atm. pressure. Most of the cyclic dimer was hydrolysed to glycine but about 25% was converted to an insoluble polyglycine. Meggy (1953)[88] studied the diketopiperazine-water system in detail at temperatures between 180°C. and 60°C. The yield of polymer at 180°C. depended on the time of heating and the proportions of the reactants. The optimum time of heating was 3 - 6 hours, and the optimum proportions 1 - 2 parts of water per 2 parts of diketopiperazine. Prolonged heating reduced the yield owing to hydrolysis of diketopiperazine to glycine, which was partially decomposed to ammonium carbonate and other unidentified products. He also determined that under the same conditions glycine and diglycylglycine did not yield polymer, but glycylglycine gave 18% of polymer. Thus he suggested the following steps for the polymerisation:-

1) diketopiperazine ——> glycylglycine.

2) glycylglycine + diketopiperazine ⇌ triglycylglycine

   or in general

   $(gly)_n$ + diketopiperazine ⇌ $(gly)_{n+2}$

3) glycylglycine ——> glycine.

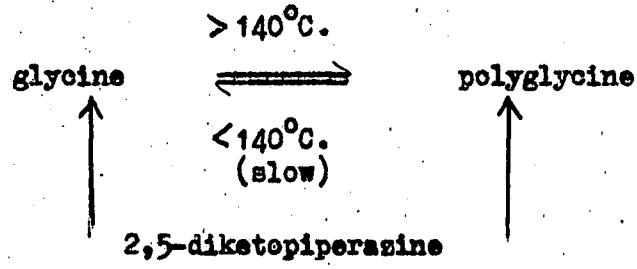
4) glycine ——> ammonium carbonate etc.

When because of reactions (1) and (3), the concentration of

dione in the aqueous phase fell below a certain value, reaction (2) was reversed, and the amount of polymer was reduced. It was not possible to test whether reaction (2) was reversible because of the steady hydrolysis, but the results of Abderhalden and Komm and of Maillard given above suggested that this was the case. The reaction was then studied at temperatures between 60°C. and 140°C. At 140°C. the reaction was complete in 2 days, at 120°C, this required 3 weeks; at 100°C. 14 weeks' heating did not complete the reaction; at 60°C. 3 months' heating produced some polymer; at 40°C. there was no sign of polymer after two years. The reaction was studied at 140°C. when the irreversible decomposition to ammonium carbonate etc. which occurred at 180°C. was inappreciable. It was shown that from 19 hours onwards the aqueous phase contained only diketopiperazine and glycine, together with a small amount of ammonia. Replacement of 10% of the diketopiperazine by glycylglycine, accelerated the initial stages of the reaction, about 40% polymer being obtained after 7 hours. But analysis of the aqueous phase showed that the degree of polymerisation of the soluble peptides was between 1.5 and 2 for the first 30 hours, showing that glycylglycine was being formed by hydrolysis of diketopiperazine as quickly as it was being consumed in the formation of polymer. A similar catalytic effect was shown

by small amounts of acids and bases. This work supported the reaction (1) in the mechanism above.

The relation between solid glycine, solid diketopiperazine and polyglycine, in the presence of water was suggested by Meggy to be:-



In the presence of an aqueous phase, diketopiperazine was unstable with respect to glycine and polyglycine at all temperatures. From the degree of polymerisation of polymers obtained from heating at 120 - 180°C., the heat and entropy of formation of the peptide bond in solid polyglycine was calculated as 3.3 kcal. mole^{-1} and 17 cal. mole^{-1} degree^{-1}, respectively. The degree of polymerisation of the products varied from 7.9 at 100°C. to 12.6 at 180°C.

D) FROM GLYCINE ESTERS

The first successful preparation of a polymer of glycine was reported by Curtius (1888)[41]. He found that if pure glycine ethyl ester was allowed to stand alone, or in solution in dry ether or dry chloroform at room temperature, diketopiperazine and a product which gave the biuret reaction

was obtained. This reaction was typical of proteins and polypeptides, the compound obtained he called Biuret base. This compound was later shown by Fischer (1906)[53] to consist principally of the ethyl ester of glycine tetrapeptide (III).

$NH_2.CH_2.COOC_2H_5$

$NH_2.CH_2.CO(NH.CH_2CO)_2NH.CH_2.COOC_2H_5$.

(III)

The relative yields of the two products were found to depend on the purity of the glycine ethyl ester, and on the mode of polymerisation. Pure dry ester standing in air gave biuret base together with 30% of cyclic dimer; in absolute ether the yield of dimer fell to only one percent.

Curtius obtained similar results using the methyl ester of glycine. Fischer and Fourneau (1901)[51] showed that when the diketopiperazine obtained by Curtius was treated with acid or alkali, for a short time, the ring was opened with the formation of glycylglycine ($NH_3^+.CH_2.CO.NH.CH_2COO^-$).

Curtius thought that the tetrapeptide ester was the end product of the reaction, but more recent work by Frankel and Katchalski (1939, 1942)[57, 58] has shown that higher peptides were formed, when the reaction times were prolonged. They used methyl, ethyl, iso-butyl, and higher esters of glycine.

They found that glycine ethyl ester, when kept at room temperature for 5 months yielded higher peptides as well as the Curtius products, and after extraction of the latter with hot water a horny insoluble material remained. This material gave a positive biuret, and ninhydrin test. Zeisel estimation of the terminal ester group gave a DP of 10 to 35. Under similar conditions the methyl ester gave polymers of higher molecular weight than the ethyl or isobutyl esters. Frankel and Katchalski (1942)[58, 59] claimed that the polymers prepared above underwent further condensation at higher temperatures, 130°C. to 150°C. to give still higher polymers, e.g. 110-glycine methyl ester or 42-glycine ethyl ester. But the work of Sluyterman and Kooistra (1952)[111] and Sluyterman and Veenendaal (1952)[113] has cast doubts on the alkoxy content estimation as a measure of the degree of polymerisation of such polymers.

The esters of glycine cannot in general be condensed to give good yields of higher peptides, a large proportion is converted into diketopiperazine. Baniel, Frankel, Friedrich and Katchalski (1948)[23] obtained a good yield of polymer having a DP of 20, by the condensation of the cetyl ester of glycine. It was not possible to detect any diketopiperazine formation in the reaction.

The rate of condensation of the esters was found to be

remarkably sensitive to experimental conditions, traces of impurity acted as catalysts. Korshak, Poroshin and Kozarenko (1954)[75] have made a detailed study of the kinetics of the polycondensation, and have studied the effects of various catalysts. They have studied the catalysing effects of carbon, dioxide, acetic acid, strong mineral acids, ethanol, peptide esters, and the carbamate. The condensation of the esters was also shown to be markedly sensitive to the nature of the solvent by Frankel and Katchalski (1942)[58, 59] and Tompa (1955)[115].

E) FROM PEPTIDE ESTERS

The dipeptide esters were not found suitable for the preparation of polypeptides. The cyclisation reaction predominates, giving an almost quantitative yield of diketopiperazine. Fischer and Fourneau (1901)[51] observed the formation of a quantity of water insoluble material giving a positive biuret reaction, when glycylglycine ethyl ester was heated at 190°C. or kept in chloroform solution at 25°C. The mechanism of the polycondensation of glycylglycine ethyl ester has been studied by Kozarenko, Poroshin, and Kus'mina (1959)[76].

The reaction leading to cyclisation of the peptide ester could be avoided by using the tripeptide or higher peptide esters. Diketopiperazine could only then be formed by chain

degradation. The condensation of tripeptide esters was first reported by Fischer (1906)[52]. He found that heating diglycylglycine methyl ester at 100°C. resulted in the evolution of methanol, and the production of the hexapeptide ester in 70% yield, and an insoluble residue of higher peptide esters of glycine. Fischer showed that the hexapeptide esters condensed slowly to insoluble higher peptide esters on prolonged heating.

Pacsu and Wilson (1942)[94] confirmed the work of Abderhalden and Fodor (1916)[2], who showed that a solution of diglycylglycine methyl ester in methanol slowly deposited the hexapeptide ester, at room temperature. The condensation ceased at this stage since the product was insoluble in methanol. The tri-, and hexapeptide esters when heated at 102° to 130°C. for 3 weeks, yielded a mixture of the tri- and hexapeptide esters respectively, and an insoluble residue of average chain length of 96 residues. The chain length was measured by a modified Zeisel method, and confirmed by titration and Van Slyke amino-nitrogen analysis. They concluded that the species present in the reaction mixture reacted only with itself, so that only peptides containing 3, 6, 12,etc., residues were formed. The evidence for this assumption was based on their claim to have obtained a pure dodecapeptide ester, but this may have been a

mixture of nona- and dodeca-peptide esters. No further evidence has come to light to support the theory, or to show that the condensation of the tripeptide esters differs from that of the simple bifunctional amino-acids, as postulated by Carothers (1931)[33] and Flory (1946)[56].

Schramm and Restle (1954)[105] working on the peptide esters of other amino acids, found peptides containing 9, 15, 18 and 21 residues in the product from tripeptide ester condensation. The molecular weights were estimated by ultra violet and infrared absorption measurements of the N-2,4-dinitrophenyl derivatives (Sanger [1945][102]). Thus the reaction followed the expected course. They also found that the peptide chains were degraded during the course of the reaction, especially on prolonged heating, to diketopiperazines. Abderhalden and Schwab (1927)[5] found diketopiperazine in the product of condensation of diglycylglycine methyl ester. This depolymerisation effect limited the size of the polymers obtained, and the presence of diketopiperazine in the product invalidated end group determination of molecular weights.

Rees, Tong and Young (1954)[100] studied the kinetics of the rate of condensation of diglycylglycine methyl ester to the hexapeptide ester, at 0°, 25° and 60°C. The reaction was found to be second order with respect to tripeptide ester. The energy of activation was 5.5 kcal. mole^{-1}. The reaction was

followed by weighing the precipitated hexapeptide ester at intervals, or titrating the residual tripeptide in solution. The rate of condensation of the hexapeptide ester was found to be very slow.

Curtius prepared triglycylglycine ethyl ester, Biuret base, and subjected it to fractional precipitation, until the basic equivalent weight corresponded to the calculated value. Heating this material at 100°C. in vacuo, produced a water insoluble product which he designated the octapeptide. Fischer (1906)[53b] prepared the corresponding methyl ester by stepwise synthesis, but found that it would not polymerise on prolonged heating at 100°C. He repeated Curtius's work and found no polymer after 8 hours heating at 100°C.

Sluyterman and Kooistra (1952)[111], and Sluyterman and Veenendaal (1952)[113] reinvestigated the polymerisation of the tri- and tetrapeptide esters of glycine. They confirmed the work of Paosu and Wilson (1942)[94], that the tripeptide methyl ester readily lost methanol at 100°C., yielding insoluble polymer, and showed that under the same conditions the tetrapeptide ester lost methanol slowly, decreasing to 0.5% after a 200 hour induction period. This verified Fischer's work. The final product contained 10% of water insoluble material (-OMe content 0.79%) which was presumed to be a polyglycine ester. The observed fall in methoxyl content

was mainly due to methyl group migration. If the tetrapeptide ester was heated at 185°C. (Mpt. is 205°C.), or in methanol at 100°C, the main product was a water insoluble polymer, and a little sarcosine, this showed that under these conditions condensation occurred more readily than methyl group migration.

The tetrapeptide esters are strikingly unreactive, under conditions suitable for the ready condensation of tri-, or hexapeptide esters. Bamford et al (1956)[20] stated that this stability might be due to a disposition of the amino and ester groups of adjacent molecules in the crystal, which would be unfavourable for reaction. Also the higher lattice energy, G_4Me has a Mpt. of 205°C., G_3Me has a Mpt. of 111°C., may be a contributing factor. The lattice forces being considerably weakened at 185°C., allows condensation of the end groups to occur, and in solution at 100° the forces are ineffective. At 100°C. the nitrogen atom of the amino group of one molecule does not approach closely enough to the carboxyl carbon atom of the adjacent molecule to effect condensation, but methyl -group migration takes place before the atoms are close enough. Methyl group migration occurrs by the methyl ester group migrating to the primary amino group, of the same molecule by an intramolecular rearrangement. The product is a zwitter ion.

$H_2N.CH_2CO.NH.CH_2CO.NH.CH_2.COO.CH_3$

triglycine methyl ester.

$\longrightarrow$ $CH_3.\overset{+}{N}H_2.CH_2CO.NH.CH_2.CO.NH.CH_2.COO^-$.

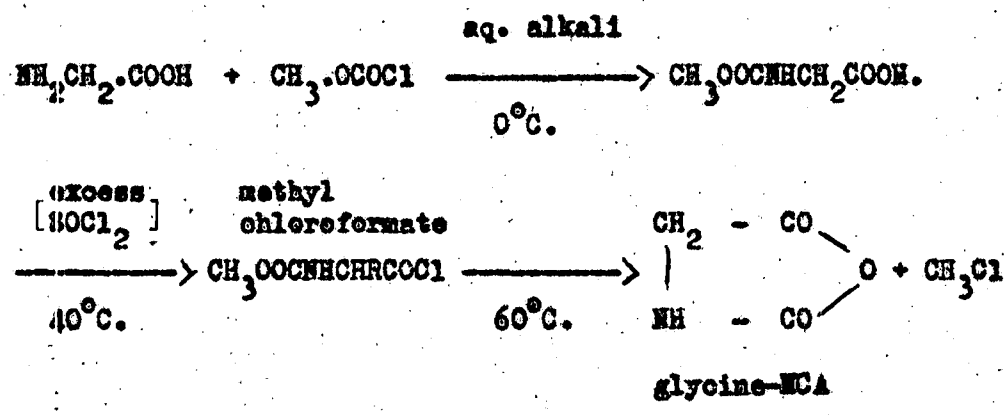
N-methyltriglycine.

This reaction invalidates the alkoxyl method of determination of the degree of polymerisation. The Van Slyke method is unreliable in this case, since it is impossible to eliminate free ethyl or methyl ester groups which would interfere. The presence of diketopiperazine in the products also render end group determinations of the degree of polymerisation inaccurate. There is also the possibility of hydantoin formation, as discussed below for N-carboxy anhydride polymerisations. This again will invalidate end-group determinations. Much doubt has been cast on the values given for the degrees of polymerisation in this section. The high values recorded for the degrees of polymerisation are probably false, since no fibre forming polymers have been obtained. The degree of polymerisation necessary for fibre formation, by comparison with the polyamides, is about 200.

γ) FROM N-CARBOXY GLYCINE ANHYDRIDE (NCA-glycine)

Leuchs and co. (1906, 1907, 1908)[80, 81, 82] prepared the N-carboxy anhydride of glycine (glycine-NCA) from glycine

by the following method:-



The reaction with methyl chloroformate in cold aqueous alkali, was essentially by the method of Fischer and Otto (1903)[54, 55]. Treatment with excess thionyl chloride at 40°C. converted the N-carbomethoxy-glycine into the unstable acid chloride. This compound underwent ring closure at 60°C. the excess thionyl chloride was simultaneously removed under reduced pressure. The product was not always crystalline, but sometimes separated as an oil. This was overcome by using phosphorus pentachloride instead of thionyl chloride. A modification by Bergmann and Zervas (1932)[25] used benzyl chloroformate, in place of methyl chloroformate. This method using phosphorus pentachloride produced better results, but the product still had to be carefully purified before use. Farthing (1950)[49] used acetic anhydride as a solvent for the reaction with thionyl chloride. The mixture was boiled for a few seconds only, he claimed a yield of 96% for glycine-NCA.

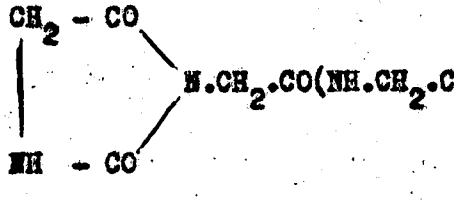
Other methods have been evolved for the preparation of glycine-NCAs:-

a) Curtius and Sieber (1921, 1922)[42] used the partial hydrolysis of malonic diester. They converted the half ester to the hydrazide and converted this to the azide, with nitrous acid in ether. The ethereal solution was then refluxed giving the NCA. This method has been modified by Hurd and Buess (1951)[71].

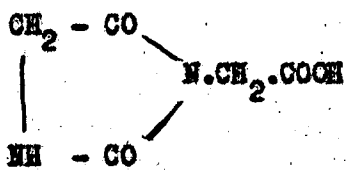
b) Fuchs (1922)[61] prepared the NCA 4-phenyl glycine by direct action of phosgene on N-phenyl glycine in cold aqueous alkali. This compound was unusually stable to water. The method was modified by Levy (1950)[83], Bailey (1950)[11], and Farthing for the preparation of less stable NCA's, including glycine NCA, by the use of dry inert solvents such as toluene, dioxane and tetrhydrofuran.

c) Farthing (1950)[49] used the disodium salt of N-carboxy glycine. This was prepared by treatment of an aqueous solution of the glycine with a molar quantity of sodium carbonate, and precipitation of the salt by methanol. The salt was suspended in ethyl acetate or dioxane and treated with phosgene or thionyl chloride giving the NCA. This has been modified by Prichard (1950)[99].

The polymerisation of glycine-NCA has been carried out in a number of ways. Above the melting point it loses carbon dioxide readily, giving polymers. Sigmund and Wessely (1926)[106], sublimed glycine-NCA without polymerisation at 50° to 130°C. and 0.5 to 10 mm. pressure. Leuchs obtained polyglycine by treating the NCA with a little water at room temperature. Wessely (1925)[117] showed that the higher the proportion of water the smaller the molecular weight of the peptides, as estimated by formol titration of the amino end groups. Becker and Stahmann (1953)[24] showed that in acid or alkaline solution glycine was the major product. At the neutral point polymers were obtained; polymerisation was most rapid in phosphate buffers, less so in citrate arsenate, and maleate buffers. Other solvents or initiators for polymerisation, are alcohol, benzene, nitrobenzene saturated with water, or organic bases. The products depend on the type of initiator and on the ratio of the concentrations of NCA and initiator. The constitution of the products from such polymerisations is doubtful. Sluytermann and Labruyere (1954)[112] claimed that the polymers had hydantoin groups at the amino end of the chains, e.g.

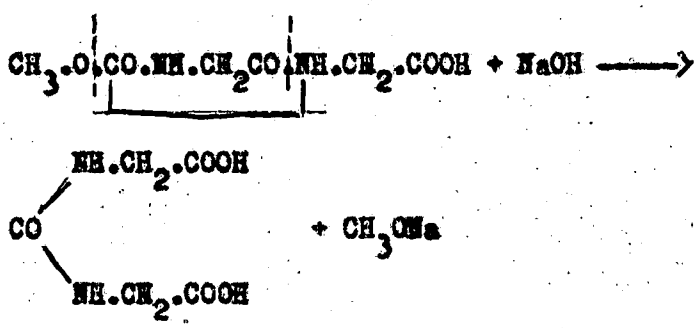


They found that the product gave colour reactions as for hydantoin compounds with picric acid and alkali. Also the acidic and basic groups in the polymer were not found to be equivalent. The evidence is not very strong but hydantoin formation is certainly possible when compounds of the type R-CO-NH-, undergo fission at the R-CO bond. Ballard, Bamford and Weymouth (1954)[14] showed that when glycine-NCA was polymerised in lithium bromide solution, hydantoin acetic acid, see below, was among the products,



Hydantoin acetic acid

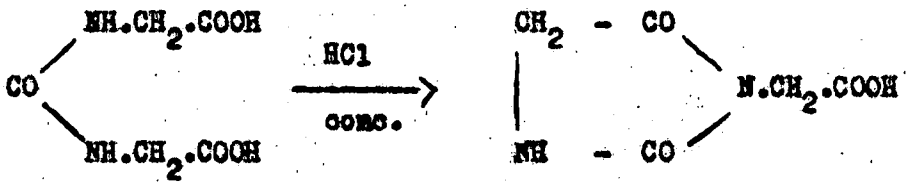
and cyclic hexapeptide. Wessely and co-workers (1952)[118] showed that the alkaline hydrolysis of carbomethoxyglycylglycine gave a urea compound.



Urea compound

This compound could have been formed only via a hydantoin compound, and could itself be converted to hydantoin acetic

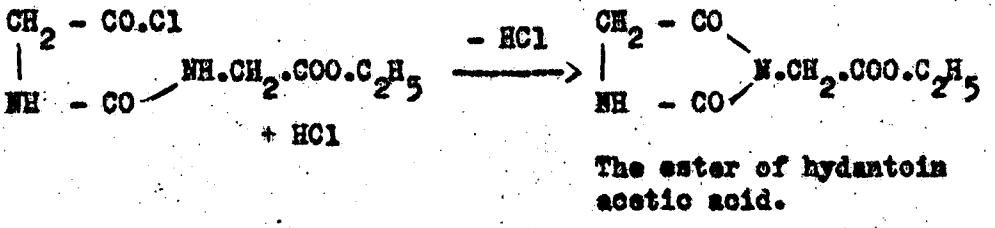
acid with concentrated hydrochloric acid.



Goldschmidt and Wick (1952)[62] showed that when glycine esters were treated with phosgene at 100°C. the isocyanate was formed, but when glycylglycine ester was so treated, the ester of hydantoin acetic acid was formed.

$$NH_2.CH_2.COO.C_2H_5 + COCl_2 \longrightarrow OCN.CH_2.COO.C_2H_5 + CO_2 + 2HCl$$

an isocyanate.



The ester of hydantoin acetic acid.

The polymerisation of glycine-NCA provides a method of obtaining high molecular weight polymers. Some control can be exerted over the degree of polymerisation of the products. The products probably however contain hydantoin terminations to the chains.

G) MISCELLANEOUS GLYCINE PEPTIDE SYNTHESES

a) Fischer and Otto (1903)[55] were able to synthesise all the glycine peptides, up to and including the hexapeptide. Glycylglycine was condensed with chloracetyl chloride in aqueous solution to form chloracetylglycylglycine (X) and on treating this compound with aqueous ammonia, chlorine was replaced by an amine group giving diglycylglycine (Y)

$$NH_2CH_2.CO.NH.CH_2COOH + Cl.CH_2.CO.Cl \longrightarrow$$

glycylglycine

$$Cl.CH_2.CO.NH.CH_2.CO.NH.CH_2.COOH$$

(X)

$$(X) + NH_3 \longrightarrow \overset{+}{N}H_3.CH_2.CO.NH.CH_2.CO.NH.CH_2.CO\overset{-}{O}$$

(Y)

Repetition of the process gave all the peptides up to the hexapeptide. Beyond the hexapeptide the polymers were insoluble in water so that this method could not be employed for high molecular weight products. It has been used in this thesis for the preparation of diglycylglycine.

b) An application of the Curtius reaction of acid azides with amines (Curtius and Sieber)[42] and (Curtius

and co. (1930))[43] was reported by Magee and Hofmann (1949)[84]. Diglycylglycine hydrazide dihydrochloride was reacted with sodium nitrite to give the acid azide addition of alkali liberated the free tripeptide azide, which condensed to a polymer of glycine.

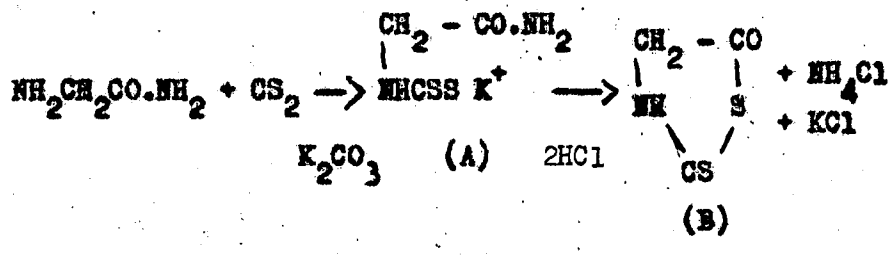
$$HCl.H(NH.CH_2CO)_3NH.NH_2HCl \xrightarrow{NaNO_2} HCl.H(NH.CH_2.CO)_3N_3 + NaCl + H_2O.$$

$$n\ H(NH.CH_2.CO)_3N_3 \longrightarrow H(NH.CH_2.CO)_nN_3 + (n-1)N_3H.$$

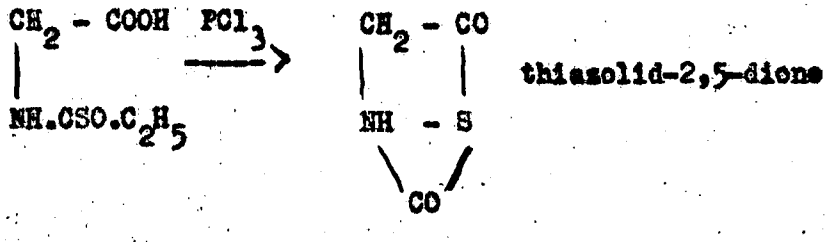
The reaction occurs since the hydrazide group reacts more readily with nitrous acid than does the amino group. This method has also been used to prepare cyclic polymers of glycine. Sheehan and Richardson (1954)[107] decomposed the azide of diglycylglycine at high dilution, they isolated a crystalline compound, in 42% yield, which they assumed was cyclo-triglycine. The similarity in properties of this compound and that obtained by Ballard, Bamford and Weymouth (1955)[13] led to a reinvestigation, which showed it to be a cyclic hexaglycine, Sheehan, Goodman and Richardson (1955)[108] and Bamford and Weymouth (1955)[22]. The latter workers also found that pentaglycylglycine azide also formed a

cyclic hexamer when allowed to decompose in dilute solution. Cyclisation occurred more readily at the hexapeptide stage than at the tripeptide stage, thus illustrating that other factors in addition to mere chain length determined the ease of cyclisation.

c) 2-thio-5-thiazolidone was synthesised by Cook, Heilbron and Levy (1948)[35]. Glycine amide was treated with carbon disulphide in the presence of potassium carbonate. Acidification of the resulting dithiocarbamate (A) gave 2-thio-5-thiazolidone (B)



They obtained a crude polyglycine by refluxing the product (B) in pyridine. The degree of polymerisation estimated by sulphur analysis and Van Slyke amino nitrogen, was about 9. A similar product was obtained by refluxing (B) in methanol, Cook and Levy (1950)[36]. The monothio analogue thiazolid-2,5-dione was prepared by Aubert, Jefferys and Knott (1951)[10], by the action of phosphorus trichloride on N-thion-carboethoxyglycine:-



thiazolid-2,5-dione

In water at 100°C. this gave an insoluble amorphous powder believed to be polyglycine.

d) Oró (1960)[93] reported the direct condensation of glycine amide to give polyglycine. The pure amide prepared by the method of Bergell and Wulfing (1910)[116] or Chambers and Carpenter (1955)[34] or commercial glycinamide hydrochloride mixed with ammonium hydroxide was used. The amide, or in the latter case the mixture, was heated along at 100°C. in a sealed vessel for 20 hours. The product was dialysed and lyophilised to remove small polymers. The average molecular weights of the products was determined by a modified Van Slyke method and gave a DP of 33. Solution of the polymer in 60% lithium bromide, aqueous solution, and titration by the Sorenson formol method, gave values for the DP of 29 to 40. The infra-red spectra showed the products to be Polyglycine.

X-RAY EXAMINATION OF THE LOWER POLYMERS OF GLYCINE

The first X-ray examination of polypeptides was carried out by Lenel (1931)[79], he examined the original preparations of Fischer, he only verified that the products were crystalline. Bernal (1931)[26] examined the crystal structures of glycine, glycylglycine, 2,5-diketopiperazine and diglycylglycine. He found that there were two crystalline forms of glycine α and β, three crystalline modifications of glycylglycine, α, β and γ, but diketopiperazine and diglycylglycine existed in only one form. The crystals belonged to the monoclinic system, except in the case of γ-glycylglycine and diglycylglycine. He also found that the diglycylglycine crystallised with 2 moles of water per mole of peptide. The diglycylglycine and γ-glycylglycine crystals belonged to the orthorhombic system.

GLYCINE

Bernal carried out a preliminary examination and showed the existance of two crystalline modifications, the α-form normal glycine and the β-form obtained by the precipitation of glycine solutions by alcohol. Hengstehberg and Lenel (1931)[68] proposed a structure for α-glycine, which has since been found to be incorrect. Albrecht and Corey (1939)[6] showed that α-glycine consisted of almost planar molecules, hydrogen bonded in sheets, they suggested that the zwitter ion structure accounted better for the hydrogen bonding, but the evidence was

not conclusive. The crystal structure has since been refined by Marsh (1958)[87]. The crystal structures of the β- and γ-glycines have been examined by Iitaka (1958, 1959, 1960, 1961)[73]. It was found that glycine had the same structure in all three modifications but it was differently hydrogen bonded to its neighbours in each.

DIKETOPIPERAZINE

Corey (1938)[38] showed the crystal structure of diketopiperazine belonged to the monoclinic system, $P2_1/a$, No. 14, Z = 2. He showed the 202 reflection was very intense, proving that the molecules must lie virtually in the 202 planes and the molecules themselves must be planar. The molecules were held together by hydrogen bonds.

GLYCYLGLYCINE

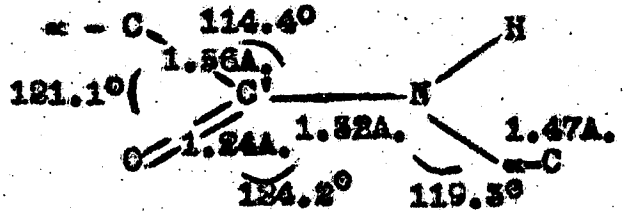
Bernal gave the unit cells and space groups of three modifications, the α-, and β- forms were members of the monoclinic system, the γ-form was orthorhombic. The β-form was examined by Hughes and Moore (1949)[70]. The crystals were shown to belong to the monoclinic system, space group A2/a, the number of moles per unit cell Z = 8. The molecules were found to have the configuration of the zwitter ion, this was established by the disposition of the hydrogen bonds. The hydrogen bonds were so arranged as to bind the structure

together, in all directions, and so dictated the mode of packing of the molecules. The molecules were coplanar except for the $\overset{+}{N}H_3$- group, which was lying 0.64 Kx. out of the plane of the other atoms.

The crystals were grown from solutions in mixtures of n-propyl alcohol and water. The α-form was always obtained at first, as plates. Repeated attempts eventually produced a few needles of the γ- form, and finally one crop of crystals were of the β-form of needles. It was not known which of the three forms was stable at room temperature, but heating the β-, or γ - form for 24 hours at 105°C. converted them into the α- form.

The bond length for the C - $\overset{+}{N}$ bond was not found to be abnormally short, Corey and Co. had obtained a value of about 1.4 A. in α-glycine and diketopiperazine. This agreed with the work of Donahue (1950)[74] who found from electron density maps that the C - N bond was normal in ($\pm$)-alanine.

Hughes and Biswas[69] found that in α-glycylglycine the carbonyl carbon atom, and the three atoms to which it was attached, were within 0.03 A. of a common plane, but the α-carbon atom was not exactly in this plane, its position corresponded to a rotation of 5 or 6° around the C' - N bond.



α-GLYCYLGLYCINE.

α-glycylglycine has one of the shortest recorded hydrogen bonds, 2.67A., and was found with a deviation (α-C - N -- O) of 22°. Again the C-N bond was normal, 1.47A.

DIGLYCYLGLYCINE

Bernal determined the unit cell and space group of diglycylglycine, and showed the existance of 2 moles of water of crystallisation per mole of peptide. The crystals belonged to the orthorhombic system and contained 4 molecules per unit cell. He only found one crystal type on precipitation with either water or alcohol.

At this point, work on the crystal structure of the lower peptides has ceased. Hughes and Biswas[69] work on the α - form of glycylglycine has not yet been published, this work was started in 1949. The structure of these peptides is by no means simple. Many difficulties are encountered working with peptides above diglycylglycine:-

a) Pure peptides can only be prepared by a stepwise synthesis, there are no direct methods of controlled polymerisation.

b) It is not easy to obtain well crystalline specimens of the peptides. The methods of preparation lead to crystalline powders, which can only be recrystallised in the form of micro-crystals. The higher one ascends the peptide series the more difficult it becomes to obtain a suitable solvent for recrystallisation.

c) Owing to the complexity of the structures, and the large unit cell, of the peptides it is not possible to obtain values for the space group and unit cell dimensions from X-ray powder photographs. The only method available is to use single crystal X-ray measurements. For this reason it was decided to grow single crystals of the ester hydrochlorides of the peptides. The ester hydrochlorides could be obtained in a chromatographically pure state. The lower members are sharp melting well crystalline materials, and they can be easily recrystallised. The structures cannot be far removed from that of the parent peptide, and would tend to approach the structure of the parent as the degree of polymerisation of the peptide increased.

DIGLYCINE HYDROCHLORIDE AND HYDROBROMIDE.

These compounds were first prepared by Kraut and Hartmann (1865)[78], and Frost (1942)[60]. They are not to be confused with glycylglycine compounds. They are molecular compounds of glycine and the glycine halide, which have crystallised out in equimolecular proportions. The general method of preparation was to dissolve equimolecular quantities of the two components in water on heating and to allow the solution to cool. A 64% yield of the diglycine hydrohalide was obtained. The melting point of the hydrochloride was 186 - 187°C.

The crystal structure of diglycine hydrochloride was investigated by Hahn and Buerger using three dimensional Patterson projection methods. The unit cell and space group were determined. The crystals were shown to be erthorhombic, with systematic absences characteristic of the space group $P2_12_12_1$, the unit cell contained 4 moles. The structure was again refined by Hahn (1960)[65]. Similar results were found for the hydrobromide, Buerger, Barney and Hahn (1956)[29]. Again the structure was refined by Hahn (1959)[64].

POLYGLYCINE

RECOGNITION THAT POLYGLYCINE EXISTED IN TWO FORMS

The analysis for elements and the determination of end groups of polyglycines had shown that the average degree of polymerisation of the polymer varied according to the means of preparation. The first X-ray study of the polyglycines was carried out by Meyer and Go (1934)[92]. They obtained X-ray powder photographs for glycine, glycylglycine, diglycylglycine, triglycylglycine, tetraglycylglycine, the penta-, hexa-, hepta- and octa-peptides and for polyglycine. They used the Curtius[15] condensation of glycine ethyl ester in chloroform to obtain biuret base, this material, they found, left a water insoluble residue, which was soluble in lithium bromide solution, and gave the X-ray diagram of polyglycine. The lower peptides were prepared by the stepwise condensation of glycine ethyl ester and acetyl glycine ethyl ester. Polyglycine was prepared by the Leuchs[80, 81, 82] carbomethoxy anhydride method. The anhydride was polymerised in the presence of water and pyridine. In both products they found free amino end groups indicating that it was not a cyclic anhydride, but a polyglycine. The product in either case showed X-ray lines analogous with those of the octa-peptide. The polymers

were not soluble in lithium bromide, sulphuric acid, or other high polymer solvents.

The X-ray diagram for the tetrapeptide showed several rings of about equal intensity and rings at 4.15 A. and 3.12 A. which were much stronger. In the case of the hexa- and hepta-peptides the 4.15 A. reflection was the strongest, the intensities were the reverse for the tetrapeptide. They suggested that the 4.15 A. line, being very intense, meant that the structure was simple, and that the atoms were arranged in planes, spaced at 4.15 A. apart. The simplest arrangement was for the chains to be arranged in a zig zag fashion in these planes, as in keratin. The products of the anhydride polymerisation were similar in their X-ray structure to the hepta-peptide. The product obtained using hot pyridine gave a different X-ray pattern, no reflection occurred at 4.15 A., instead reflections appeared at 4.4 A. and 3.45 A. This could be dissolved in lithium bromide solution, and precipitated by water. This product gave the more usual X-ray pattern showing a strong 4.15 A. ring.

The material which Meyer and Go obtained from the NCA and hot pyridine, showing strong reflections at 3.45 A. (V(s) and 4.4 A. (Ves.), has become known as Polyglycine I. The product which they obtained from solution of Polyglycine I

in lithium bromide solution, showing reflections at 4.15 A. (v.s.) and 3.1 A.(m), has become known as Polyglycine II. The material which they called heptaglycylglycine, appears from the photograph to be a typical mixture of Polyglycine II, and a little Polyglycine I. The hexaglycylglycine photograph shows the rings for Polyglycine II only.

The existance of the two forms of polyglycine was forgotten for about 14 years. Astbury and coworkers (1948)[9] and Astbury (1949)[8], revived interest in the polyglycines. They repeated Meyer and Go's work, and found that polymerisation of glycine-NCA in hot pyridine or ethyl acetate gave Polyglycine I. They proposed a structure for Polyglycine I, see later. The results suggested that Polyglycine I had an extended β-configuration. Bamford et al (1955)[17] put forward possible structures for Polyglycine II and determined that this could only be obtained by precipitation of solutions of Polyglycine I. They also pointed out that Meyer and Go's Polyglycine shown in plate II was a mixture of Polyglycine I and II. Crick and Rich (1955)[39] also proposed a structure for Polyglycine II. All the postulated structures were based on the X-ray powder photographs which show only three or four not very sharp rings.

Meggy and Sikorski (1956)[91] examined both forms of Polyglycine. The Polyglycine I, was prepared by the

polymerisation of glycine in hydrochloric acid. The Polyglycine II was prepared by dissolving the Polyglycine I in a saturated aqueous solution of calcium chloride, and precipitating by water at 20°C. Examination under the electron microscope of the Polyglycine II crystals showed them to be almost hexagonal. Slow precipitation of the most soluble portion of the Polyglycine II resulted in thin hexagonal leaflets, showing growth steps. The height of the steps was estimated from the length of the shadow on the micrograph, and was found to be less than the 40 A. calculated for a polymer of DP = 12.2, but this was the DP of the bulk polymer, and not the soluble fraction. The growth steps were due to the occurrence of dislocations in the crystal, and was to be expected from the non-homogeneous nature of the polymer. The densities of the polymers were determined and it was found that the density increased as the number average degree of polymerisation increased. Thus two samples of Polyglycine I showed DP's of 8.4 and12.2, the measured densities were 1.469 and 1.486 gms. per ml. respectively. The Polyglycine II, having a DP of 10.6, had a density of 1.475 gms. per ml.

The only other published work on the dimorphism of

Polyglycine was the work of Oró (1960)[93] who obtained mixtures of the Polyglycines from the polymerisation of glycinamide (see methods of preparation of polyglycine).

PROPERTIES OF THE POLYGLYCINES

The Polyglycines are both white infusible powders. They are insoluble in the common hydrogen bond breaking solvents, such as phenol, water, dimethylformamide and formic acid. They are soluble in concentrated mineral acids, including phosphoric acid, and in saturated aqueous solutions of lithium chloride, bromide, iodide and thiocyanate. Similarly in saturated calcium chloride and thiocyanate, in zinc chloride, and in sodium nitrate. The Polyglycine I is least soluble, the mixture has to be heated to help solution, whereas Polyglycine II will dissolve at room temperature. Precipitation of solutions of either form gives Polyglycine II only. Meggy and Sikorski (1956)[91] claimed that if a solution of Polyglycine I in aqueous saturated calcium chloride was precipitated at temperatures between 60°C and 100°C by water, a mixed product containing both polymers was obtained. Below 60°C Polyglycine II was obtained alone. This has now been checked and no trace of Polyglycine I has been found at any temperature between 20°C and 120°C under a wide variety of precipitation conditions. Meggy and Sikorski also stated that if the type II polymer was heated in water at 140°C, it was partially converted to type I. This conversion has been confirmed and shown to occur at any

temperature between 60°C and 160°C in a variety of solvents besides water. They also stated that Polyglycine II was probably a metastable form, this has now been confirmed.

Densities:-

The densities were determined by Meggy and Sikorski:-

Polyglycine type	I	I	II
No. average DP	8.4	12.2	10.6
Density gm/cc	1.469	1.486	1.475

showing that the density increased with the DP.

Crystal shapes:-

The crystal shapes were examined under the electron microscope by Meggy and Sikorski. Polyglycine I consisted of flat parallelograms, of axial ratio 0.9, and acute angle of 70°. Polyglycine II showed crystals which were almost true hexagons.

Infrared Spectra

The infrared spectra of the two forms of Polyglycine were studied by Elliott (1953)[47] and Elliott and Malcolm (1956)[48]. The original work gave bands at 1648 cm^{-1}, 1632 cm^{-1} (C-O stretching bands), 1558 and 1521 cm^{-1} (N-H deformation) and 3293 and 3301 cm^{-1} (N-H stretching) for Polyglycine I. It was later realised that the first value given in each set was due to Polyglycine II present as an

impurity. Polyglycine I, the β-form, was obtained by casting films from dichloracetic acid or trifluoracetic acid. The wave numbers of the infrared absorption bands, as well as dichroism in a partially oriented sample, coupled with the presence of a strong 1.16 A. X-ray reflection, (Bamford et al (1953)[18]) indicated an extended chain. The Polyglycine II, precipitated from lithium bromide, gave no 1.16 A. reflection and was certainly not fully extended in its structure. (The complete spectra are shown opposite). The N-H stretching band for Polyglycine II, at 3290 cm^{-1} and the weak accompanying band, at 3095 cm^{-1}, were much stronger than in any other synthetic peptide. The wave number of the C=O band in Polyglycine II was much higher than in the β-structure, although the value of 1630 cm^{-1} was low and was only found in β-polypeptides.

A band was found at 1685 cm^{-1} in Polyglycine I; this was thought by Ambrose and Elliott (1951)[7] to be a folded form of Polyglycine present together with the β-form. Removal of the small peptides, discovered by Ballard and co. (1954)[14], reduced the intensity of this line, but it was certain that the line is due to Polyglycine I, (Bamford et al (1956)[21]). The band could be made to disappear on conversion of the polymer to Polyglycine II. They also stated that the band could not be made to reappear, on reconversion of the Polyglycine II into

form I, as it could not be due to impurities. No details were given of the method used for the conversion. The existance of the two forms of Polyglycine, and the presence of impurities probably explain the differences in the spectra observed by Hurd, Bauer and Klotz (1953)[72] and Blout and Linsley (1952)[27], and the above workers. Blout and Linsley considered that the 1015 cm^{-1} band was characteristic of the diglycyl group.

Absorption of dyes etc.

Bamford, Boulton, Manby and Ward (1954)[16] showed that Polyglycine absorbs acid, basic, direct and dispersed dyes. The polymer was Polyglycine prepared from glycine-NCA, and may have been a mixture of both forms. It was found that the absorption of acid dyes was only 61% of the apparent amino group content, by Van Slyke determination. Also if the polymers were acetylated, the dye uptake was reduced by about the same amount as the Van Slyke amino group content. They concluded that some of the end groups in the polymer were inaccessible to both the dye molecules and the acetylating mixture. Meggy and Sims (1956)[90] observed a similar effect for polymers prepared from 2,5-diketopiperazine. Polyglycine I absorbed only 42% of the theoretical quantity of Orange II,

and heating the polymer reduced this to 13%. Polyglycine II absorbed Orange II equivalent to the total terminal amino groups in the polymer, and this absorption was not affected by heating. They also showed that the dyed material had the same X-ray diffraction pattern as the undyed material, for both polymers, and no dye diffraction lines appeared. Thus the dye taken up did not alter the crystal structure. The theory that dye was absorbed only in the amorphous regions of textile fibres, see Speakman et al (1946)[114], could not be applied in the case of the highly crystalline Polyglycine II. The dye must have been absorbed in crystalline regions of the polymer. The low dye uptake of Polyglycine I is exceptional, since nylon, wool, silk and other insoluble proteins have been found to combine with acid dyes in amounts corresponding to the content of basic groups. There is no explanation for this peculiar behaviour.

Chemical reactivity of Polyglycines

Apart from the formation of an acetyl compound mentioned above, there is no record of any derivatives of either Polyglycine II or I having been prepared. The major difficulty in attempting any reactions involving the polymers, is the finding of a suitable polymer solvent, the only useful

ones are concentrated acids, or salt solutions. A possible compound with cuprammonium solution has been prepared in this thesis.

THEORIES OF THE STRUCTURES OF THE POLYGLYCINES

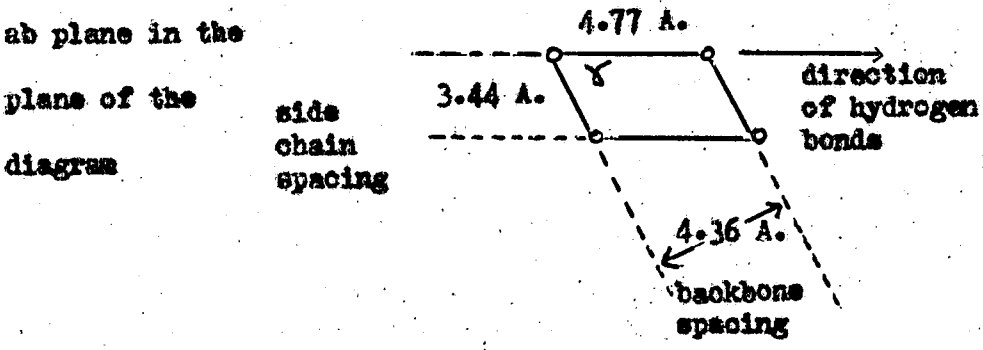
The crystal structures of the Polyglycines have not yet been determined because it has not yet been possible to obtain well crystallized specimens. Polyglycine II can be crystallized in hexagonal microcrystals, but Polyglycine I cannot be reprecipitated from solution as such. All the crystal structure work which has been carried out has been based on X-ray powder photographs and infra red spectra. This has resulted in much intelligent guess work with little experimental evidence to back it up.

Meyer and Go (1934)[92] suggested that in the Polyglycine II type structure, since the 4.15 A. reflection was so strong, that the chains were arranged so that the atoms lay in sheets spaced at 4.15 A. apart.

POLYGLYCINE I

Astbury and co. (1948)[9] studied the powder photographs of Polyglycine I. The polymer was that obtained from glycine-NCA and hot pyridine. They assigned Polyglycine I a tentative rectangular cell, a = 4.36 A. (or twice this value), b = 3.44 A., c (the fibre axis) = 7 A.approximately. This was later revised by Astbury (1949)[8] to accord with the work of Bunn and Garner (1947)[31] on hydrogen bonding in polyamides. They found that the distance between hydrogen

bonded chains was 4.77 A. Astbury found that the interplanar spacing in Polyglycine was 4.36 A., this was considerably less than the chain separation which was probably close to that in the polyamides. He assumed therefore that the hydrogen bonds were not normal to the sheets of chains but were inclined as shown:-



The angle $\gamma = \sin^{-1}[4.36/4.77] = 66^{\circ}$. Taking the fully extended residue length as 3.67 A. [as determined by Corey (1940)[37]] Astbury calculated the density of Polyglycine I to be 1.56 gm. per cc. This value agreed fairly well with the measured value of 1.51 gm. per cc. Pauling and Corey (1953)[97] calculated the intensities in a powder photograph of Polyglycine I based on the assumption that the chains had an antiparallel pleated-sheet configuration and that the ab plane was that postulated by Astbury. They reported that the agreement between the observed and calculated values of the intensities, was good enough to assign the given

structure to Polyglycine I. They gave no details of the calculations.

Bamford and co. (1953)[18] pointed out that the diffraction pattern of Polyglycine I showed a spacing at 1.16 A. A reflection occurred at this spacing in other polypeptides and silks, and was shown to be the 006 reflection (c was the molecular chain axis), thus if Polyglycine I consisted of extended polypeptide chains a strong reflection would be expected at or near this spacing, whether the chains were packed in a rectangular or triclinic cell.

The structures proposed were surprising, owing to the large value obtained for the fibre identity period. If Pauling and Corey's (1953)[97] value of 7.23 A., derived from measurements on small peptides, was accepted, then the chains in the polymer could not be fully extended. If the bond angles or distances in the polymer were different from those in small peptides, then the fibre period may be near to 7.0 A and the chains fully extended. The fibre period did not correspond to the pleated sheet value of 6.68 A. and according to the ideas of Pauling and Corey (1951)[102] the structure would represent an unstable configuration. The most important factor in determining the stability was the staggering of the bonds. In the light of work on β-poly-L-alanine, Pauling and Corey

(1953)[95] suggested that bond staggering was not as important, and put forward two additional pleated sheets with identity periods of 7.0 A. (antiparallel) and 6.5 A (parallel). The period of the former agreed closely with the observed repeat in Polyglycine I. Thus it appeared that the chains in Polyglycine I were not fully extended, but were slightly collapsed from the extended structure, as suggested by Bamford and co (1956)[20]. The packing of chains was essentially similar to that in β-poly-L-alanine, β-poly-γ-methyl-L-glutamate, and Bombyx and Tussah silk. The basic unit was that shown by Astbury (1949)[8] and the separation of hydrogen bonds was in every case between 4.7 and 4.8 A. but the other dimensions varied from polymer to polymer.

POLYGLYCINE II

The structure of Polyglycine II was first investigated by Bamford et al (1955)[17]. They found that Polyglycine II did not show a spacing in its X-ray diffraction pattern corresponding to 1.16 A. The two forms of Polyglycine could not therefore have arisen from different ways of packing extended or nearly extended chains. They concluded from this that the chains in the two forms must have had different configurations. They found that the chains in Polyglycine II could not be fully extended, since this would have required the 4.15 A. line to be the 100 reflection, and the next strongest 3.1 A. line, the 010 reflection, in order to obtain a reasonable molecular packing. The predicted intensities for this arrangement were found to be much different from the observed values. The 4.15 A. spacing appeared to be due to planes which were parallel, or nearly so, to the fibre axis. They found that films made by dissolving the polymer in a mixture of formic acid and calcium chloride, and then drying off the formic acid, could be rolled producing some orientation of the Polyglycine II. X-ray investigation of these films showed that the 4.15 A. spacing was probably equatorial rather than meridional. Hot rolling of the films which were shown to contain no β-material, produced a small

amount of the doubly oriented β-form. The mean direction of the chain axis was found to lie in the direction of rolling. The high intensity of the 4.15 A. reflection showed that the electron density had a very marked periodicity at 4.15 A. They concluded that this was the separation of layers of polypeptide chains, as did Meyer and Co. They also stated that an hexagonal array was possible, but excluded the α-helix, which would have given hexagonal packing, since the close approach of chains in this structure would have given an impossible value of about 3 gm. per cc. for the density. They examined the two most likely structures of those proposed for polypeptide chains, they were the 2.2_7 helix, and the unrotated parallel polar sheet. (Ref. Donahue (1954)[46], Pauling and Corey (1951)[95]). Both of these structures were proved unsatisfactory, since neither would give an X-ray pattern which agreed with that observed. They did suggest that in the case of the 2.2_7 helix, that a triclinic cell was possible, but suggested that this could not be verified until a fibre pattern could be obtained. From the work on the rolling of films, they suggested that the molecules of Polyglycine II were folded, and that on rolling a number were pulled out into the

β-form. The case presented showed strong evidence for a configuration of the polypeptide chain in Polyglycine II, which was outside the then accepted range of structures.

The accepted structure of Polyglycine II was proposed by Crick and Rich (1955)[39]. They proposed that all the polypeptide chains in the structure were parallel, and each one had a threefold screw axis. The chains were packed in an hexagonal array, each chain being hydrogen bonded to each of its six neighbours. These hydrogen bonds lay roughly perpendicular to the screw axis, and ran in several directions, and not merely in one direction as in the β-structure.

The projection along the x-axis and perpendicular to the screw axis, the z-axis runs vertically. The residue at the bottom of the figure shows the peptide group inclined at about 35° to the fibre axis, having its plane perpendicular to the plane of the paper. The hydrogen bonds run in an infinite series from one cell to the next, perpendicular to the paper, joining residues of adjacent chains. The atoms of these residues all lie on an inclined lathe. There is a second such series of lathes 3.1 A. higher in the structure, but these run at an angle to the paper owing to the 120° rotation of the screw axis. At 3.1 A. higher the lathes are again repeated, again at an angle to the paper. Above this the structure is repeated. The crystallographic description given by Crick and

Rich was a = 4.8 A., c = 9.3 A., the space group was $P3_1$, containing one residue per asymmetric unit. The calculated density was 1.54 gm. per cc., the observed density according to Bamford was 1.43 gm. per cc., and according to Meggy was 1.475 gm. per cc. This was not very good agreement, but Crick and Rich pointed out that the calculated density was often 5 - 10% higher than the observed value. The structure was built according to the standard Pauling and Corey[97] bond distances and angles. The hydrogen bond distance, nitrogen to oxygen, was a little short at 2.76 A., but was acceptable according to Donahue (1952)[45]. The hydrogen bond was almost straight. Since it contained no asymmetric carbon atom, they pointed out that the mirror image structure, space group $P3_2$ was equally possible. They calculated all the spacings and intensities expected from the structure, and found a good qualitative agreement with the photographs obtained by Bamford and co. The very strong 4.15 A. reflection they assigned the index $10\bar{1}0$. The 3.1 A. reflection was mainly due to $10\bar{1}2$, spacing 3.09 A. with a small contribution from 0003, the strength of the reflection was considered to be due to the inclined planes of the peptide groups making up the lathes. They point out that although they are confident that this is the structure, or the structure is closely related to the given structure, there is a family of possible structures of this

general type. All these would have in common polypeptide backbones with threefold screw axes, and a repeat of about 9.34. Examination of a structure in which one of the chains was withdrawn and replaced, running in the opposite direction, showed that the structure would have a similar X-ray pattern to that obtained. They state that only a very detailed structure study would enable one to say whether the given structure, which appeared to be the simplest, was correct or only a first approximation.

Crick and Rich determined that only Polyglycine could form such a lattice, since there is no room for side chains in the structure unless some of the hydrogen bonds are broken. They did not see any reason why a single Polyglycine chain should take up this structure, and concluded that it must be dictated by the interaction between neighbouring chains, which would impose an exact three fold screw axis. They thought that the reason why a fibre photograph had not been obtained was probably that any process to produce orientation would tend to stretch the structure and it would then pass over to the more extended β-form of Polyglycine I.

THE α-HELICAL FORM OF POLYGLYCINE

No other forms of Polyglycine have been discovered. In particular no one has found the α-helix form which has

been observed for other polypeptides and proteins. Bamford and co.[17] stated that the only evidence which supported the existance of such a form was the observation of parallel dichroism of the NH-band in a specimen which had been stretched in polystyrene, Bamford and co (1953)[18]. More recent observation had suggested that this may have been a "cross β-structure", since the C=O band accompanying the dichroic NH-band had the β-rather than the α-frequency.

EXPERIMENTAL

SECTION.

THE SEARCH FOR METHODS OF PRECIPITATING POLYGLYCINE I FROM SOLUTION.

SUMMARY OF RESULTS.-

The investigation was carried out with the aim of obtaining good crystals of Polyglycine I, and also to see whether any other forms of Polyglycine could be found. It has not been found possible to obtain well defined crystals of Polyglycine I by this means, and no new forms of Polyglycine have been found. The investigation has provided information about the relative stabilities of the two forms of Polyglycine.

Solutions of Polyglycine I in aqueous saturated calcium chloride solution have been precipitated by a wide variety of precipitants. The use of water as a precipitant resulted in Polyglycine II being obtained, at all temperatures between 15°C. and 120°C. This did not confirm the work of Meggy and Sikorski[91](1956), who found that if precipitation was carried out at temperatures above 60°C., the product was a mixture of Polyglycine I and Polyglycine II. Below 60°C. they claimed that only Polyglycine II was obtained, and at 100°C. only Polyglycine I was precipitated. The reaction has been fully investigated over a wide range of precipitating

conditions, but in all cases only Polyglycine II has been obtained.

Precipitation of the Polyglycine I solution in calcium chloride solution has also been carried out, using, methyl and ethyl alcohol and glycerol as precipitants. In these cases the major part of the precipitated polymer was Polyglycine II, but the X-ray photograph of the product showed that it also contained a little Polyglycine I. Varying the conditions of precipitation had little effect on the proportions of the two polyglycines obtained. Methyl alcohol used as the precipitant produced the most easily separated product, and this also contained the highest proportion of Polyglycine I, as judged from the intensities of the X-ray lines. The ethyl alcohol gave a product which was a sticky mass, and was very difficult to separate from solution. Glycerol gave an easily separated product, but the Polyglycine I X-ray lines were not very intense.

Precipitations of the polymer solution in calcium chloride solution by dimethylformamide, and by 0.88-ammonia solution, were found to produce only Polyglycine II.

Aqueous zinc chloride(70%) was used as a solvent for Polyglycine I. Precipitation of this solution

by water was carried out at various temperatures. The results were identical with those obtained for solutions in calcium chloride. Polyglycine II only was obtained at all temperatures.

Precipitations of solutions of Polyglycine I in zinc chloride solution by the rapid addition of ethyl alcohol in large excess gave a product which showed ill defined X-ray lines for Polyglycine I only. An X-ray diffractometer trace taken at a later date confirmed that this material was ill defined crystals of Polyglycine I containing only very little Polyglycine II. The mixing of the alcohol and the polymer solution resulted in the evolution of much heat. No such heat evolution was noticed in the case of precipitation by water. Precipitation by mixtures of alcohol and water and by the slow addition of alcohol resulted in mixtures of both Polyglycines being obtained.

Solutions of Polyglycine I in concentrated acids, such as hydrochloric acid, sulphuric, nitric, or phosphoric acids, when precipitated by water, produced only Polyglycine II. Similar results were obtained on precipitation of solutions of Polyglycine II in these media.

Cuprammonium hydroxide was also found to be a

solvent for the Polyglycines. It was found that some form of Polyglycine-cuprammonium complex could be precipitated from the solution by ethyl alcohol. The X-ray photographs showed that the products obtained from the two forms of Polyglycine differed in the intensity of one X-ray line. A blank precipitation of cuprammonium hydroxide solution by ethyl alcohol, confirmed that the precipitates obtained were true complexes and not mixtures of the Polyglycines and precipitated cuprammonium hydroxide derivatives. The alcohol precipitated products were decomposed by dilute sulphuric acid. The product from Polyglycine I showed that considerable change had occurred, the polymer had been changed to a mixture of Polyglycine I and Polyglycine II. This is evidence that the structure of Polyglycine I is at least partially retained in this particular solution. The residue from Polyglycine II, gave Polyglycine II only on decompostion of the complex.

Polyglycine I and II were both dyed a pale blue colour, by copper ions, on boiling with a saturated aqueous solution of copper sulphate. The polymer did not dissolve in the solution. The X-ray photographs of the dyed products showed no lines for copper. The product from Polyglycine II showed a

weak line at 3.45 A. characteristic of Polyglycine I. Heating Polyglycine II in the saturated copper sulphate solution at 72°C. for 24 hours produced no change in structure. Thus the conversion of the Polyglycine II into Polyglycine I must have been temperature dependent. The Polyglycine I obtained from a solution in zinc chloride by precipitation by alcohol, was also boiled with the copper sulphate solution. The polymer became dyed a deeper blue than in either of the above cases. This seemed to indicate that more sites were available in this for coordination with copper ions. The absorption of copper ions was in all cases reversible, the copper could be removed by warming with dilute sulphuric acid. No change was observed in the X-ray structure of the Polyglycine I, ex zinc chloride solution, on the removal of the copper ions.

DISCUSSION OF RESULTS.-

The only successful method for the precipitation of Polyglycine I from the solutions of Polyglycine, was that using zinc chloride solution as the polymer solvent, and ethyl alcohol as the precipitant. This gave only ill defined crystals , and could not be made to produce good crystals of Polyglycine I. Even this material contained a little Polyglycine II. The poor quality of the product seems to indicate that precipitation of crystal nuclei occurs rapidly, and that the rate of nucleation greatly exceeds the rate of growth of the crystals. The actual time taken for a precipitate to appear was as long as 10 minutes. This seems to indicate that the Polyglycine is present in solution as a metastable form, this will be shown to be Polyglycine II, and that the thermal energy released by the heat of mixing of the solutions; is sufficient to accelerate the conversion to the stable Polyglycine I. The alternative theory is that the strucutre is partially retained in solution. This theory would not account for the precipitation of a 10% solution of Polyglycine II, by alcohol, giving Polyglycine I as the product. All other precipitating agents produce Polyglycine II only from solutions of Polyglycine II, except

methanol which produces a mixture of both forms of Polyglycine.

The transition reported by Meggy and Sikorski (1956) was definitely not observed. The conditions were sufficiently varied that if such a transition had existed it would have been discovered. The claim that a transition temperature, in the region of 60°C. exists for the two forms of Polyglycine is refuted. Evidence will be presented to show that Polyglycine II is a metastable form and that no true transition occurs between the Polyglycines.

In the cases of the two polymer solvents dealt with above, the high heat of mixing or dilution, is unique to the precipitants methanol and ethanol. No such strong heat evolution is observed with the other precipitants, all of which give Polyglycine II only, or at best only a little Polyglycine I. It is of interest that Polyglycine II is much more soluble in mineral acids, and dissolves much more easily than Polyglycine I. This would be expected if Polyglycine II were the metastable form.

The formation of a complex of both Polyglycines with cuprammonium hydroxide is also interesting. The only compound of Polyglycine which has been reported, is a partially acetylated polymer, Bamford et al.[16](1954).

The X-ray photographs, showed that the structures of the complexes from both Polyglycines were very similar, the lines only differing in intensity and not in position. The decomposition of the complexes showed that the Polyglycine I structure had been partially retained in the complex, but part of it had been converted into Polyglycine II. This means that the Polyglycine I structure is partially retained in solution in cuprammonium hydroxide, at least. It is not possible to say at which stage the partial change occurs. A further investigation would be necessary to ascertain the point of conversion.

The heating of the Polyglycines in saturated copper sulphate solution, resulted in the partial conversion of Polyglycine II into Polyglycine I. This also supports the idea that Polyglycine II is the metastable form, also it suggests that the conversion is temperature dependent, although the copper sulphate may act as a catalyst . If the Polyglycines are heated for any length of time in any of the salt solutions or acids used, they are substantially degraded, thus it is impossible to tell if conversion has occurred.

The evaporation of solutions of the Polyglycines

in saturated aqueous calcium chloride solution, produced yellowish white deliquescent solids. These solids were found to be X-ray amorphous, and so the polymers were assumed to be present in solid solution in hydrated calcium chloride. No complex corresponding to the cuprammonium complex could be obtained on precipitation of the two Polyglycines in zinc chloride or calcium chloride solutions by alcohol.

EXPERIMENTS.-

The Polyglycine I used in these experiments was prepared by the direct polymerisation of glycine [89]. The polymerisations were carried out in hydrochloric acid. The proportions of glycine to 10 normal acid were 5 gms. to 1 ml.. Polymerisations were carried out in sealed tubes heated at 140°C. for 24 hours. The polymer obtained was washed free of acid with water, washed with alcohol, and dried in a vacuum desiccator.

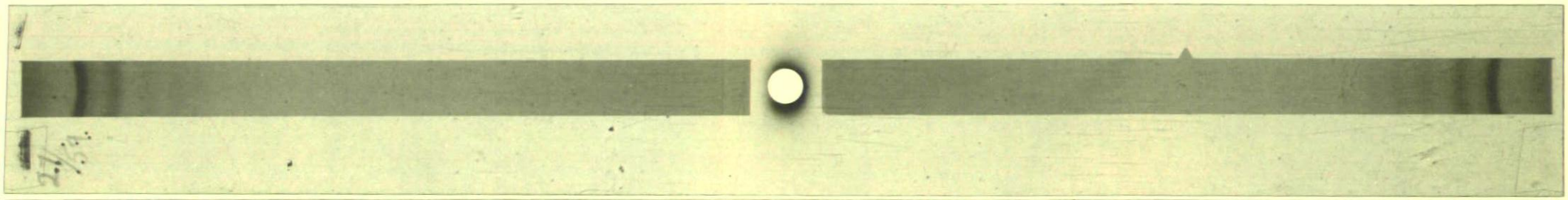
1) PRECIPITATION OF POLYGLYCINE FROM SOLUTIONS IN SATURATED AQUEOUS CALCIUM CHLORIDE.

a. Precipitation at different temperatures.

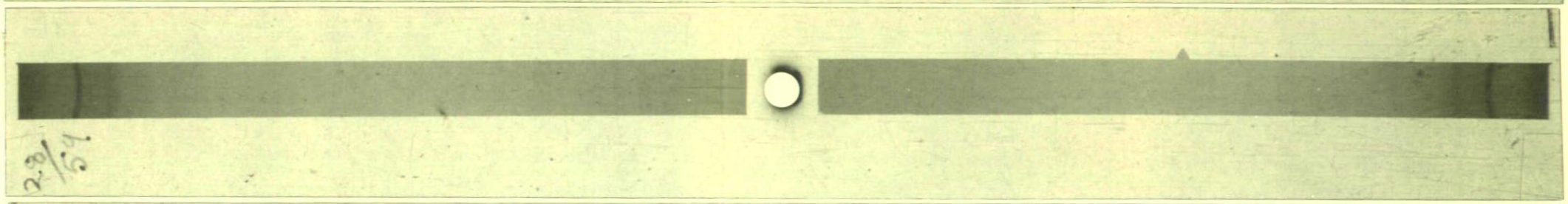
A ten percent solution of Polyglycine I in saturated aqueous calcium chloride was prepared. 5 ml. portions of this solution were placed in centrifuge tubes in a water bath , initially at 30°C.. These solutions were precipitated by the addition of 5 ml. portions of distilled water, the mixture was well stirred during and after precipitation. Further precipitations were carried out at 10°C. intervals between 30°C. and 90°C.. A final precipitations was carried out at 99°C. The precipitated product was centrifuged down, in each case, washed with water until free of calcium

PRECIPITATION OF A 10% POLYGLYCINE L SOLUTION IN SATURATED CALCIUM CHLORIDE SOLUTION, BY WATER, AT TEMPERATURES BETWEEN 22°- 99°C.

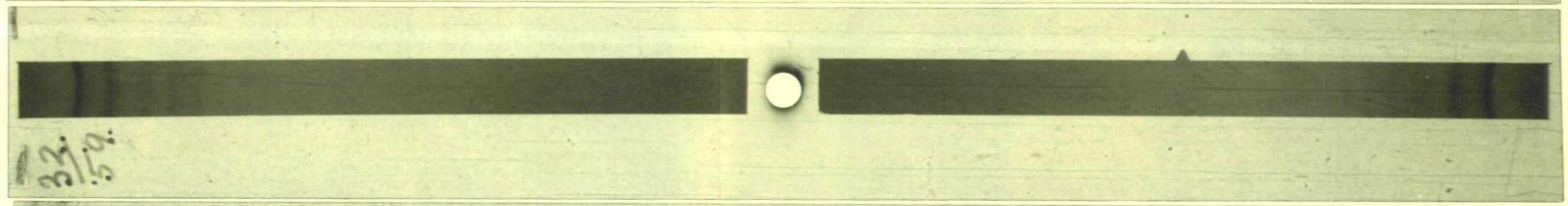
22°C.



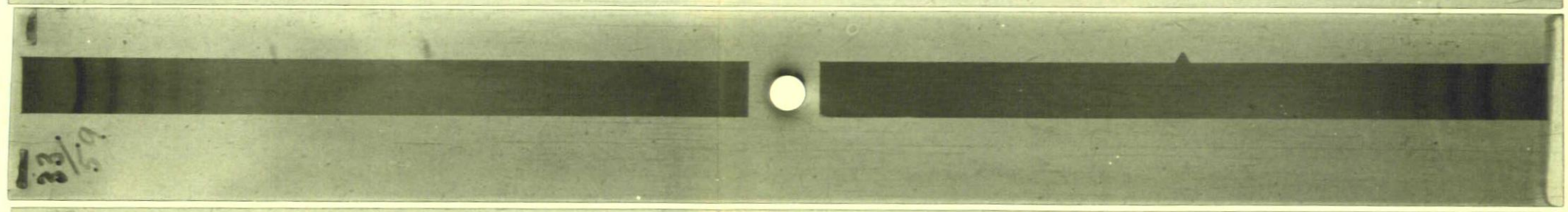
40°C.



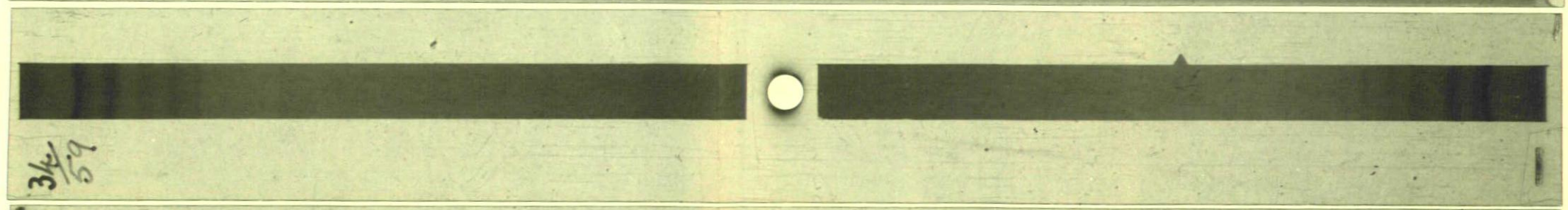
50°C.



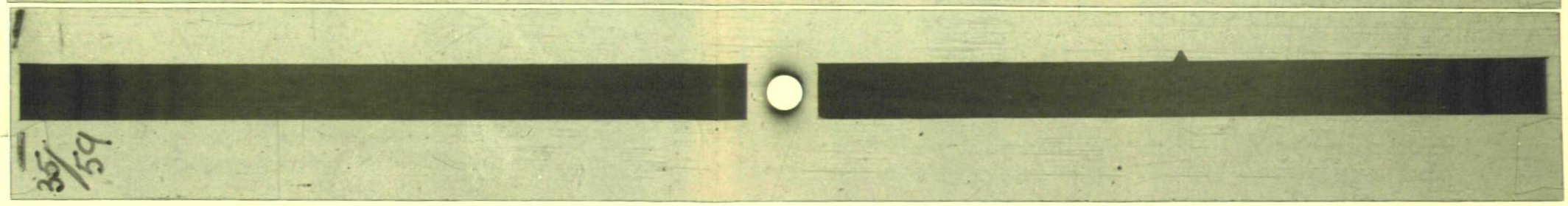
70°C.



80°C.



99°C.



chloride, washed with alcohol, and dried over calcium chloride under vacuum. An X-ray powder photograph of each specimen was taken. All the photographs were identical, and showed only the spacings for Polyglycine II, no Polyglycine I lines appeared.

b. Precipitation to different dilutions.-

A further set of precipitations of a 10% solution of Polyglycine I in calcium chloride, was carried out at 100°C.. This time 5 ml. portions of the polymer solution were precipitated by 5, 10, 25, 50, 75, and 100 ml. portions of distilled water. (i.e. dilutions of 1:1, 1:2, 1:5, 1:10, 1:15, 1:20.). It was thought that the concentration of calcium chloride, and its ratio to the concentration of water might have affected the precipitation. But again only Polyglycine II was obtained.

c. Precipitation from solutions containing lower concentrations of Polyglycine.

A 5% solution, weight for volume, of Polyglycine I in saturated calcium chloride solution was prepared. 5 ml. portions of this solution were precipitated, at 100°C., with varying volumes of water, as before. Again all the products showed the X-ray pattern characteristic of Polyglycine II only.

When a 5 ml. portion of the solution was precipitated with 5 ml. of water, there was a 20 minute delay before a precipitate appeared. The precipitate obtained showed the "leafing effect" markedly. This effect is characteristic of platelet crystals, e.g. clays. On cooling the solution this effect was masked by further precipitation. The X-ray photograph showed no sharper lines in this case, when compared with the other products. It appeared that the initial precipitate was of better crystalline quality than the bulk of the precipitate. All the tubes were kept in the water bath at 100°C. for 45 minutes to ensure that precipitation was completed at 100°C.

d. The approximate solubility of Polyglycine in calcium chloride solution.

A 5 ml. portion of 10% polyglycine in saturated aqueous calcium chloride required 3.1 ml. of water to commence precipitation. The water water was added slowly over a period of hours to allow for any delay in precipitation. Thus at the point of precipitation the solution contained about 28% of calcium chloride by weight.

A saturated solution of Polyglycine I in saturated aqueous calcium chloride was then prepared . It was found that the solubility of the Polyglycine was approximately 210 gms. per litre of saturated calcium chloride. 5 ml. of this solution was precipitated by 5 ml. of water at 100°C.. This again showed only the X-ray diagram of Polyglycine II.

e. Precipitation above 100°C.

10 ml. of 10% Polyglycine in saturated calcium chloride was placed in a test-tube, and this was lowered into a wide Carius tube containing 10 ml. of water. The tube was carefully sealed, and placed in an oven at 110°C, and allowed to reach this steady temperature. The tube was then inverted to allow the two solutions to mix, and precipitation to occur. The product from this precipitation was also Polyglycine II only. This was repeated with a 5% solution, and a saturated solution of the polymer. Polyglycine II was the only product.

f. Preparation of a possible Polyglycine and calcium chloride complex.

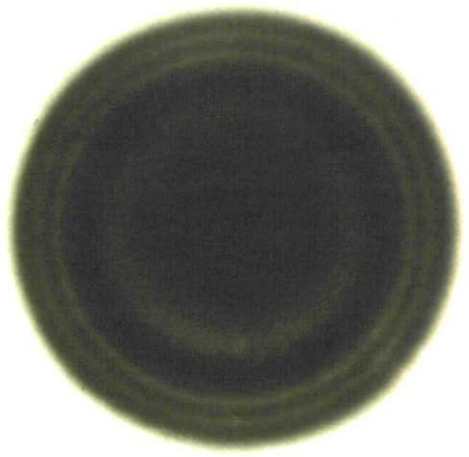
A 10% solution of polymer in calcium chloride

was left standing in a boiling water bath. As the water evaporated off the solution became more viscous, until finally a precipitate was thrown down. It was then allowed to stand and cool. The precipitate was centrifuged off. The supernatant liquid was diluted with water but no polymer was precipitated. Some of the paste like residue was x-rayed, the photograph was devoid of any lines. The remainder of the paste was stirred with water, it dissolved at first, but then a slight precipitate showing some "leafing" was obtained. This gave the X-ray pattern of Polyglycine II.

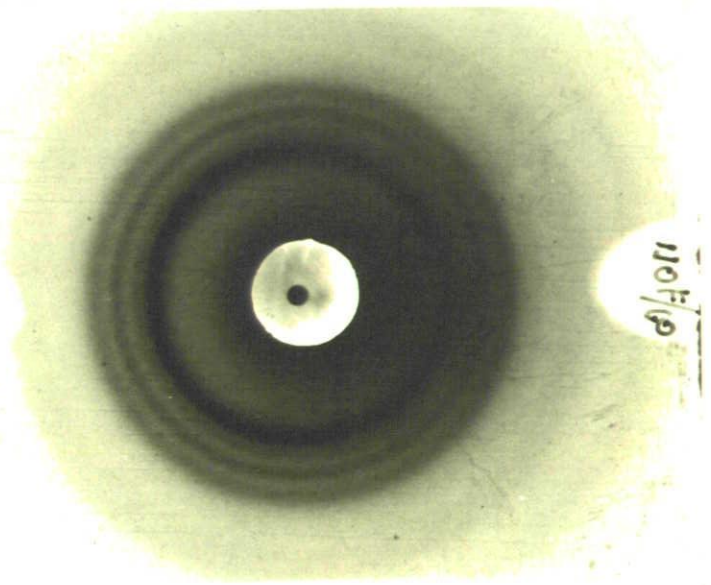
2) PRECIPITATIONS OF SOLUTIONS OF POLYGLYCINE IN CONCENTRATED AQUEOUS CALCIUM CHLORIDE SOLUTION, BY SOLVENTS OTHER THAN WATER.

i. Precipitation by ethyl alcohol.

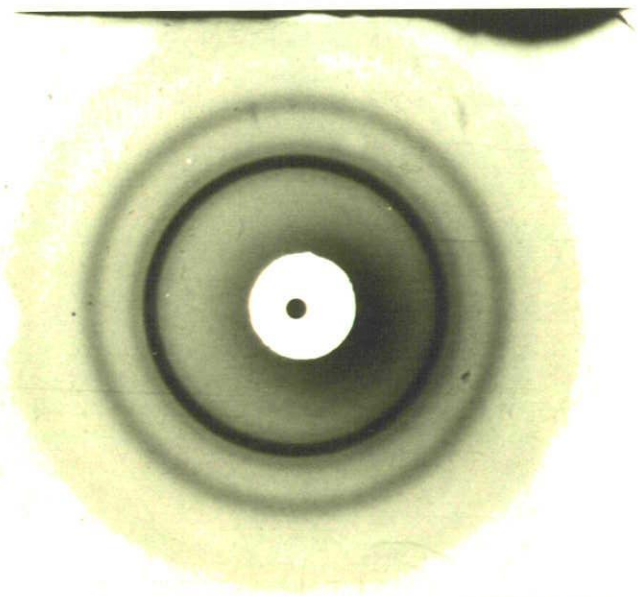
A 5% solution of the Polyglycine in calcium chloride solution, was precipitated by 25 ml. of ethyl alcohol. This was found to be the minimum quantity of ethanol which would induce precipitation. It was also found best to add the polymer solution to the alcohol; otherwise a gummy mass was obtained, which stuck to the stirring rod. The solid was centirfuged down to a gummy mass. This residue



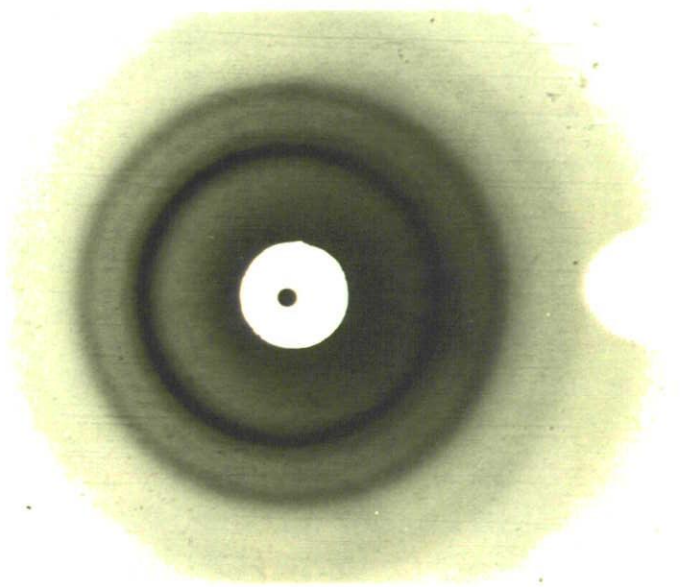
5% POLYGLYCINE I (5ml)
IN AQUEOUS SATURATED
CALCIUM CHLORIDE SOLUTION
PRECIPITATED BY 25ml OF
ETHANOL.



5 m.l 5% POLYGLYCINE I
IN SATURATED AQUEOUS
CALCIUM CHLORIDE SOLUTION
PRECIPITATED BY 25 ml. OF
METHANOL



5 m.l. 5% POLYGLYCINE I.
IN SATURATED AQUEOUS
CALCIUM CHLORIDE SOLUTION.
PRECIPITATED BY 5 m l. OF
METHANOL.



5 m.l. SATURATED POLYGLYCINE I
IN SATURATED AQUEOUS
CALCIUM CHLORIDE SOLUTION
PRECIPITATED BY 25 m.l. OF
METHANOL

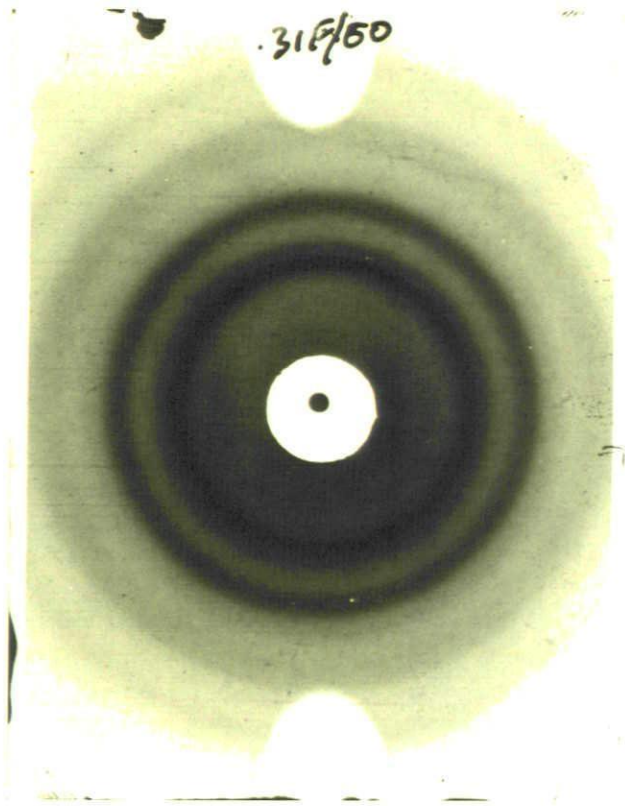
gave an X-ray diagram showing only a diffuse ring at about 4.15 A.. It was not stable, but decomposed in the air. This may have been a Polyglycine and calcium chloride complex.

A 5 ml. portion of a 5% solution of the polymer was treated with 50 ml. of ethanol. There was a voluminous precipitate, part of which dissolved on washing with water. This left a fine white residue of about one quarter of the initial bulk. The X-ray photograph showed lines for both Polyglycine I and II. All attempts to obtain pure Polyglycine I in this way gave only mixtures of both forms; variations of the concentration and the temperature up to 50°C. were tried. This method was abandonned because of the unsatisfactory nature of the precipitates.

ii. Precipitation by methyl alcohol.

5 ml. portions of 5% polymer in saturated calcium chloride was easily precipitated by the addition of 5 ml. of methanol. The product was a fine white powder. An X-ray photograph showed it to be Polyglycine II only. A further 5 ml. portion of the polymer solution was treated with 25 ml. of methanol. The X-ray photograph of the precipitated product showed strong Polyglycine II lines, ans only weak Polyglycine I lines.

Exmx.



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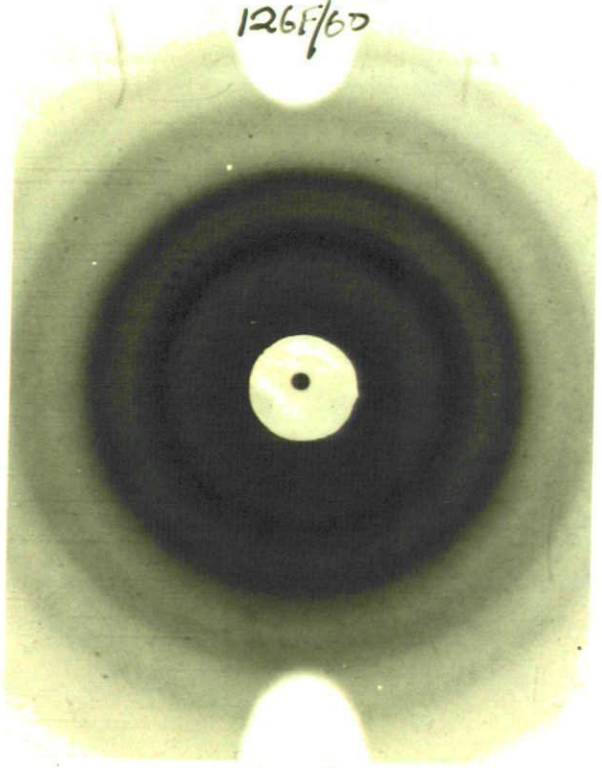

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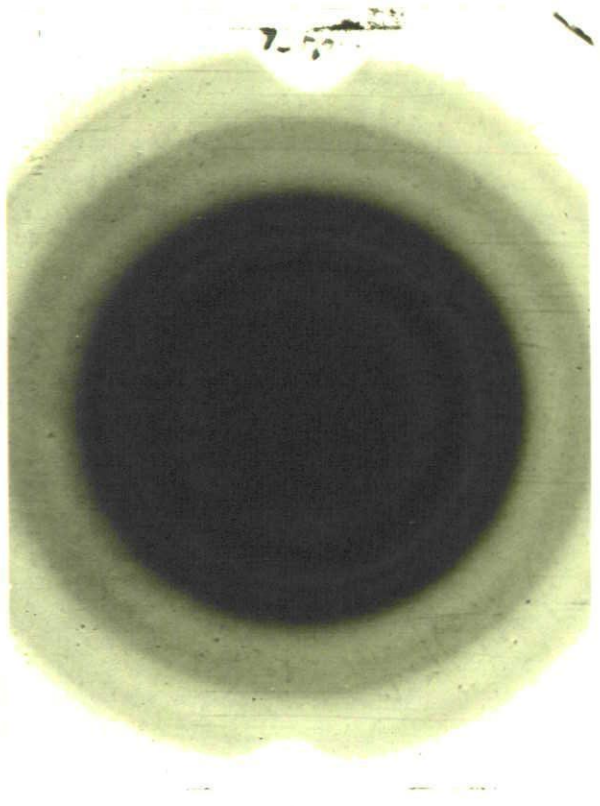

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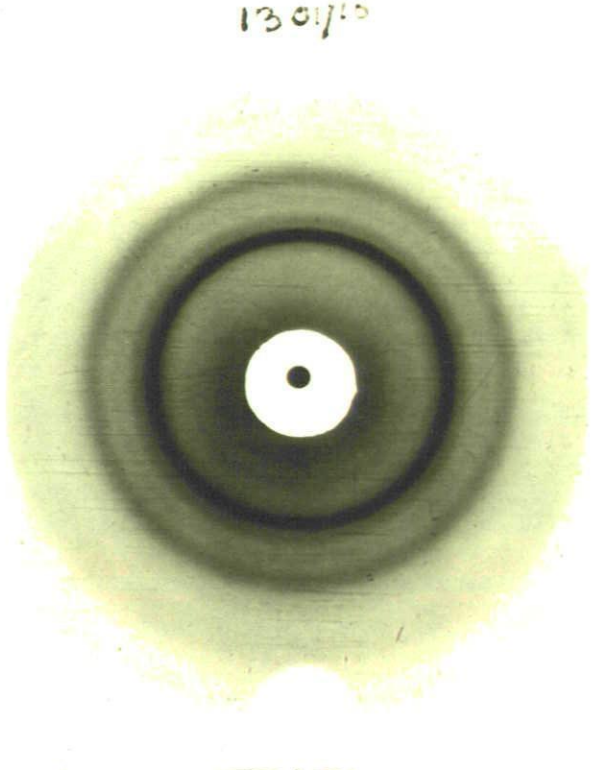

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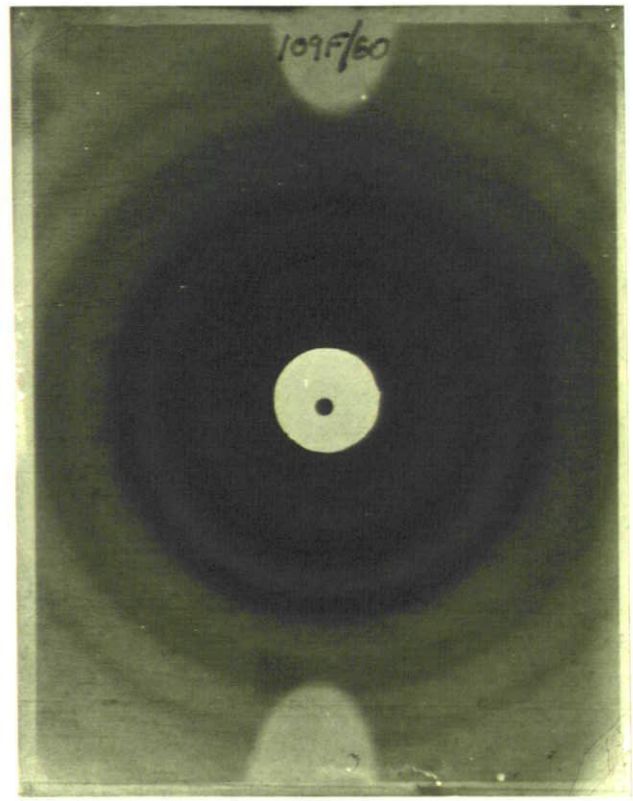


1

PRECIPITATION OF SOLUTIONS OF POLYGLYCINE I IN SATURATED AQUEOUS CALCIUM CHLORIDE SOLUTION BY GLYCEROL. (5 m.l. PORTIONS)

1. 5% SOLUTION + 10 m.l. GLYCEROL
2. 10% SOLUTION + 25 m.l. GLYCEROL
3. SATURATED SOLUTION + 10 m.l. GLYCEROL
4. " " + 15 m.l. "
5. " " + 25 m.l. "

A 5 ml. portion of a saturated polymer solution was precipitated by 25 ml. of methanol. This again, gave a product showing weak Polyglycine I X-ray lines, and strong lines for Polyglycine II.

The product obtained in this reaction was much easier to separate, than in the case above. There was no tendency for gum formation. The relative intensity of the X-ray lines, indicated that it did not produce so great a proportion of Polyglycine I, as precipitation by ethanol.

iii. Precipitation by glycerol.

A 5 ml. portion of a 5% polymer solution was precipitated by 10 ml. of glycerol. The precipitate was washed with alcohol, and dried in vacuo, over calcium chloride. The X-ray photograph showed lines for Polyglycine II only.

5 ml. portions of a saturated polymer solution were precipitated by 10, 15,, and 25 ml. of glycerol respectively. The X-ray photographs showed that addition of 10 ml. precipitated Polyglycine II only. In the remaining cases, the 3.45 A. line for Polyglycine I appeared but was very weak. All the precipitations were carried out at room temperature.

This method again did not produce sufficient Polyglycine I to warrant further investigation.

iv. Precipitation by dimethylformamide.-

Both 5% and saturated solutions of Polyglycine were precipitated by 10, and 20 ml. portions of dimethylformamide at room temperature. The products were washed with water and alcohol. The X-ray diagrams in both cases showed only Polyglycine II lines.

v. Precipitation by 0.88-ammonia solution.-

Precipitations of 5 ml., of 5%, polymer solutions were ~~xxxx~~ carried out, using 5 ml. of 0.88-ammonia solution at room temperature. The product was always Polyglycine II only.

2) PRECIPITATION OF POLYGLYCINE FROM SOLUTIONS IN SEVENTY PERCENT AQUEOUS ZINC CHLORIDE SOLUTION.

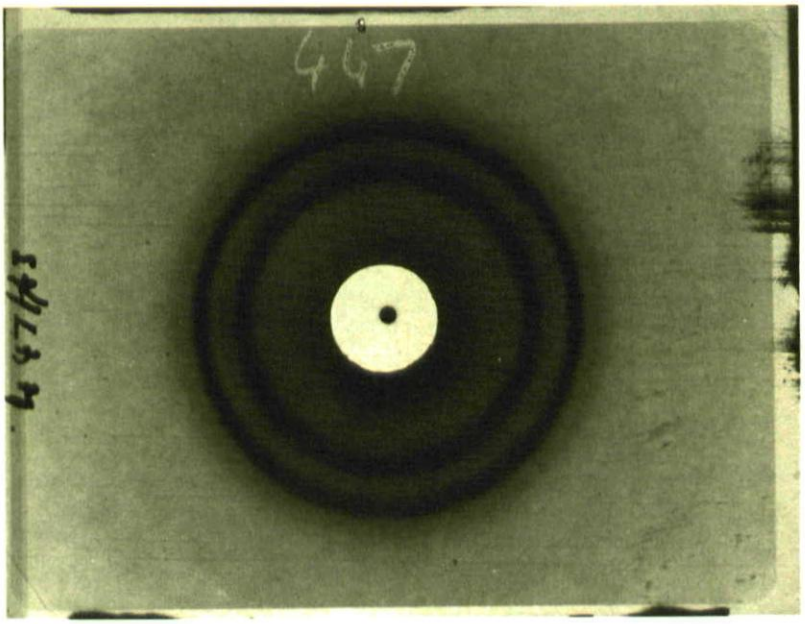
a. Precipitation by water.

A seventy percent solution, weight for weight, of zinc chloride in water was prepared. This was used to prepare a 5% solution of Polyglycine. 5 ml. portions of this solution were treated with various volumes of water. It was found that the ration of

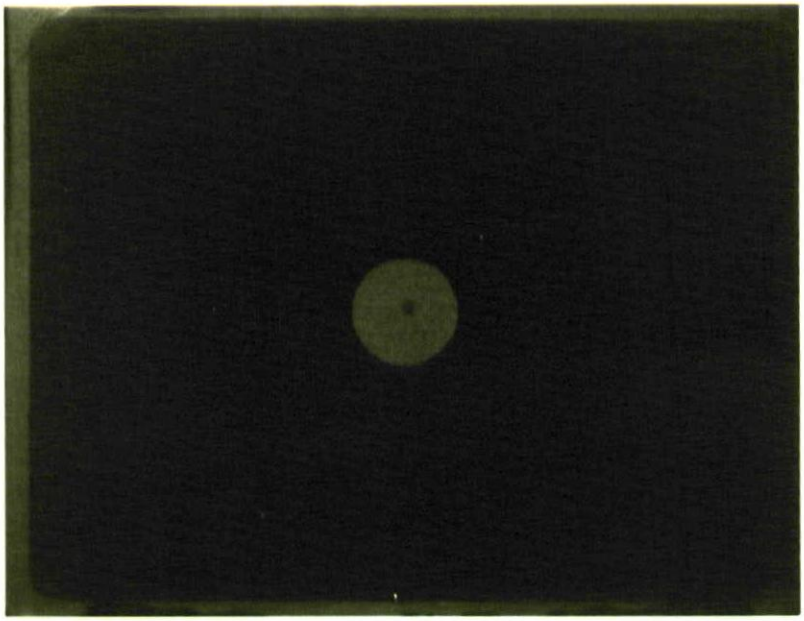
concentrations of zinc chloride to water was 1:31, at the point of precipitation. A precipitate was obtained on standing overnight at 18°C.. 35 ml. of water was found to produce almost complete precipitation of 5 ml. of the polymer solution. The ratiox of concentrations of zinc chloride to water was then 1:64. Precipitation was carried out at 18, 40, 60, 80 and 100°C.. In all cases the product obtained was always Polyglycine II only. The precipitation at 100°C. required addition of more than 30 ml. of water for completion.

b. Precipitation by ethyl alcohol.-

5 ml. portions of the polymer solution in zinc chloride solution, were precipitated by 5, 10, 15, 20 and 25 ml. portions of ethanol. No precipitate was obtained in the first two cases, but heavy precipitates were obtained in the last two cases. All the precipitations were carried out at 20°C.. The X-ray photographs of the products, showed that the samples precipitated by 15 and 25 ml. of ethanol were not Polyglycine II. The lines were characteristic in position and relative intensity of those expected



5 m l 10% Polyglycine I
solution in 70% aqueous
zinc chloride solution
precipitated by 40 m l
of industrial spirit.



5 m l. 5% Polyglycine I.
Solution in 70% aqueous
zinc chloride solution
precipitated by 30 m l.
of industrial spirit.

for Polyglycine I. A blank precipitation of the zinc chloride solution by alcohol produced only a very slight precipitate on long standing. The samples obtained were pale yellow powders. The products are undoubtedly Polyglycine, since they are soluble in strong salt solutions only. The X-ray lines are not very strong nor very sharply defined, thus the samples are not very well crystallised.

A 10% Polyglycine solution was found to give similar results, to the 5% solution. It was found that for a 5 ml. portion of polyglycine solution, rapid addition of 60 ml. of ethanol produced only Polyglycine I. If the alcohol was added slowly in small quantities, the product contained some Polyglycine I, but was mostly Polyglycine II. It was noticed that on adding the alcohol the solution became quite hot, i.e. a high heat of mixing. Addition of small quantities of ethanol, less than 20 ml., led to coagulation of the precipitate. In these cases the product was always a mixture of Polyglycine I and II. Dilution of the alcohol with water before addition to the polymer solution,

resulted in only Polyglycine II being obtained. Similarly addition of alcohol and then water resulted in mixtures of both forms being obtained, with a predominance of Polyglycine II. This happened unless the addition of water had been delayed until considerable precipitation had occurred. The heat of mixing on adding water alone is not so great as that produced on adding alcohol alone, or alcohol and water mixtures.

The Polyglycine I obtained by this method, was of very poor crystalline quality, this can be seen from the diffractometer trace (Chart 4, at the end.) The trace also showed the presence of small quantities of Polyglycine II as well. The poor quality suggests the rapid growth of crystal nuclei from solution, the rate of nucleation far exceeding the rate of growth of the nuclei. The actual time taken for visible precipitation to occur was as long as ten minutes. This seemed to indicate that the Polyglycine was present in solution as the metastable Polyglycine II. The thermal energy released on mixing with alcohol, accelerated the conversion to

the stable Polyglycine I. High local heating on mixing produces rapid nucleation, but is not sufficiently sustained to encourage growth of the nuclei.

A solution of Polyglycine II(10%) in aqueous 70% zinc chloride solution was made up. 5 ml. of this solution was precipitated by 40 ml. of alcohol, the product was Polyglycine I, again much heat was evolved and precipitation was slow. Precipitation of this solution with mixtures of alcohol and water gave Polyglycine II as before. Precipitation by methanol gave a mixture of both forms of Polyglycine.

The precipitation of a solution of Polyglycine II by alcohol to give Polyglycine I, means that the conversion of form must have occurred on precipitation by the alcohol. The solution of the polymer could not have contained Polyglycine I nuclei, unless they were formed during the preparation of the Polyglycine II solution in the zinc chloride. This not likely since if the formation of a solution required the conversion of Polyglycine II (solid) to Polyglycine I(in solution), then the Polyglycine I would be more easily dissolved in the zinc chloride than the Polyglycine II. The observed facts are that Polyglycine II is almost twice as

soluble, and much more easily soluble, in strong salt solutions, than Polyglycine I(see Sec. Exp. 11.).

3) PRECIPITATION OF POLYGLYCINE FROM SOLUTIONS IN CONCENTRATED MINERAL ACIDS.

a. Hydrochloric acid.-

An approximately 10% solution of Polyglycine I, in concentrated hydrochloric acid, was made up in the cold, solution was very slow. 5 ml. portions of this solution were precipitated by both ethanol and water. The reactions were carried out at room temperature and at 100°C. In all cases the product gave the X-ray diagram of Polyglycine II only. There was no Polyglycine I in the products, not even those precipitated by alcohol. These experiments were repeated for a 10% Polyglycine II solution in hydrochloric acid. The solution of this polymer was much easier, and it was much more quickly dissolved. The products of precipitation were again only Polyglycine II.

b. Nitric acid.-

A 10% solution, approximately, of Polyglycine I in nitric acid was prepared. The solution required heating to dissolve the polymer. These 5 ml. portions of this solution were precipitated by water at room

temperature and 100°C.. The product was Polyglycine II,only. The precipitate showed a fair "leafing effect," but the X-ray lines were no sharper than usual. The acid had attacked the polymer and greatly decreased the yield of product.

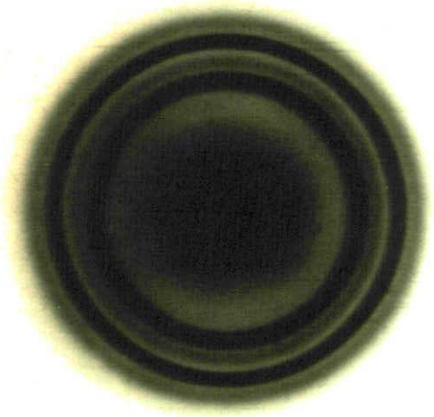
c. Sulphuric acid.-

An approximately 10% solution of Polyglycine I in concentrated sulphuric acid was prepared. The polymer had to be heated to facilitate solution, thus the yields on precipitation were low, owing to acid attack of the polymer. The solution was precipitated at room temperature and 100°C.. The product was Polyglycine II only.

4) PRECIPITATION OF THE POLYGLYCINES FROM SOLUTIONS IN CUPRAMMONIUM HYDROXIDE.

A solution of cuprammonium hydroxide was prepared by the aeration of metallic copper in 0.88-ammonia, thus avoiding the presence of sulphate ions in the solution.

It was found that Polyglycine II was about twice as soluble as Polyglycine I in the cuprammonium hydroxide solution. Precipitations of these solutions



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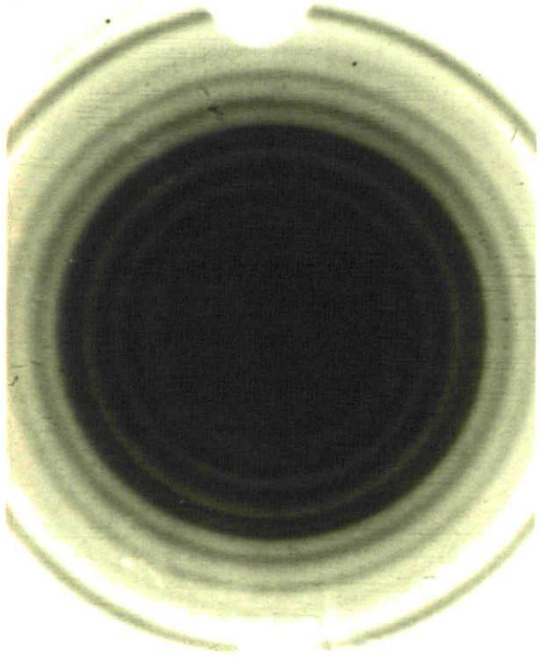

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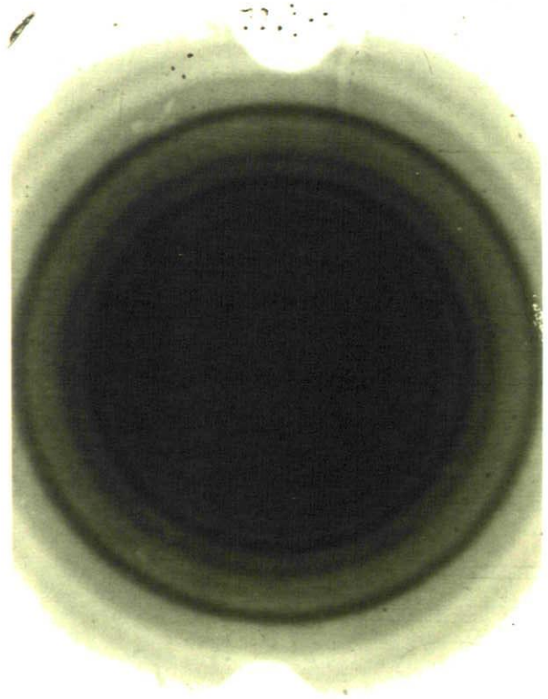

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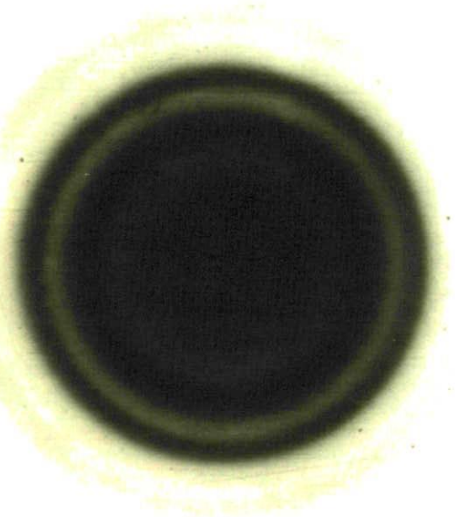

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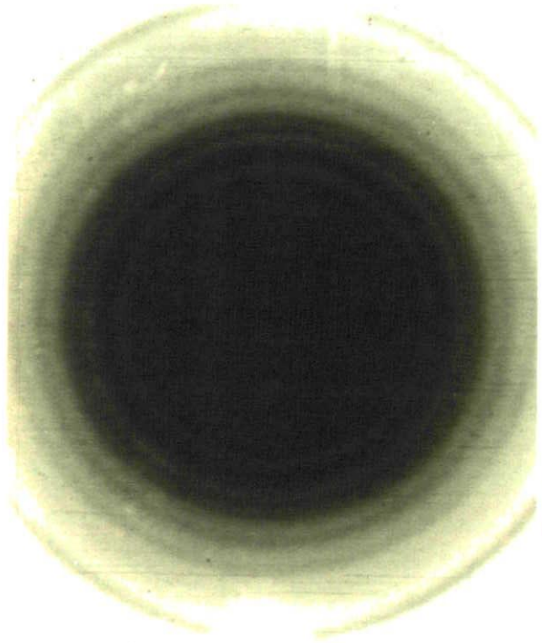


3

SOLUTION OF THE POLYGLYCINES IN CUPRAMMONIUM HYDROXIDE SOLUTION.

1. POLYGLYCINE I/CUPRAMMONIUM SOLUTION PRECIPITATED BY ETHANOL.
2. RESIDUE ABOVE, TREATED WITH DILUTE SULPHURIC ACID.
3. POLYGLYCINE II/CUPRAMMONIUM SOLUTION PRECIPITATED BY ETHANOL
4. RESIDUE ABOVE TREATED WITH DILUTE SULPHURIC ACID.
5. CUPRAMMONIUM SOLUTION PRECIPITATED BY ETHANOL.

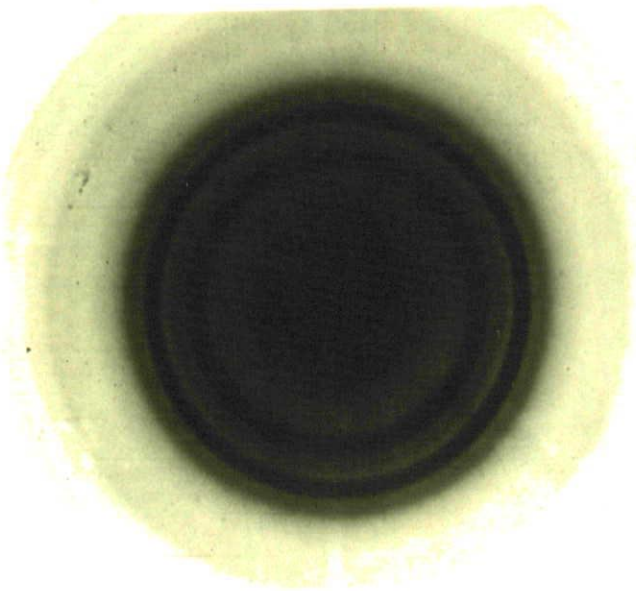
by alcohol gave blue-purple coloured precipitates. The X-ray diagrams of these precipitates showed that the positions of all the lines were identical for both Polyglycine I and Polyglycine II derived products. The only difference was in the intensity of the third ring from the centre. This ring was much stronger on the photograph of the precipitate from Polyglycine I and cuprammonium solution. A blank precipitation of cuprammonium hydroxide solution by alcohol gave a green product. The X-ray diagram of this product showed a number of lines which are not present on the other photographs. There are some lines common to ~~both~~ all three photographs however. The line which differed in intensity on the precipitate photographs was coincident with the very strong line of Polyglycine II. (4.15 A.). The next line outward from this corresponded to the strong line for Polyglycine I (3.45A.), this line was common to all the photographs. The medium strength line of Polyglycine II(3.1 A.) was a part of the doublet which occurred as the next lines moving outward on the pattern. This doublet occurred on both precipitate photographs but was

stronger in that from Polyglycine I. No such doublet appeared on the blank photograph. Comparison of the polymer photographs with that of the blank, showed that the polymer containing precipitates, were not merely mixtures of the Polyglycines and the compound precipitated by the addition of alcohol to cuprammonium solution. The blank photograph showed many different lines , from the other two photographs. These lines differed markedly in both position and intensity, from lines on the other photographs. Thus the Polyglycines had formed compounds with the cuprammonium hydroxide.

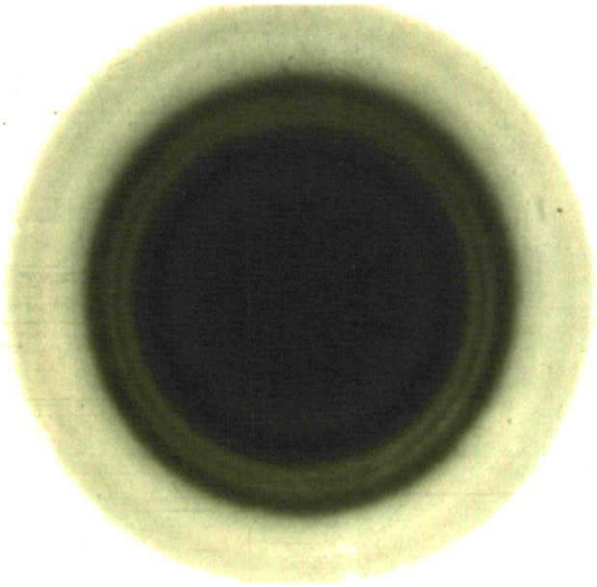
The precipitate obtained from the solution of the polymers in cuprammonium solution was then decomposed by the addition of dilute sulphuric acid. This left a white residue in both cases. The residues were washed with water and alcohol and dried over calcium chloride in vacuo. The X-ray photographs for each sample were taken. The residue from the precipitate which had initially contained Polyglycine I, was considerably changed. It now contained both Polyglycine I and Polyglycine II. The residue from the precipitate which had initially contained Polyglycine II, was unchanged after decomposition of the complex. These reactions were carried out on

a qualitative scale, the polymer solutions being about 5%, weight for volume, in the cuprammonium solution. Each solution wasprecipitated by about 30 ml. of ethanol. The precipitates were decomposed by 5 ml. of 2N. sulphuric acid. Attempts to repeat the reactions on a quantitative scale, did not produce well crystalline samples. The X-ray photographs showed only weak diffuse lines.

Saturated solutions of both Polyglycine I and Polyglycine II in the cuprammonium solution were prepared at room temperature. 5 ml. portions of these solutions were precipitated at room temperature, by 25 ml. portions of ethanol. The X-ray photographs obtained for these precipitates were identical. They were also identical with the photographs obtained in the quantitative experiment above. All the photographs showed one intense, but diffuse, line at 3.45 A.(the outer ring for Polyglycine I.). The remaining weak lines were lower Bragg angle, longer spacihgs. These precipitates were then decomposed by 5 ml. of 2N. sulphuric acid. Some polymer was precipitated, but in too small a quantity for an X-ray photograph. For the purpose of this investigation



0·5 gm. POLYGLYCINE I
+ 5 m.l. SATURATED
AQUEOUS COPPER SULPHATE
SOLUTION, HEATED OPEN AT
72°C, FOR 24 hrs



0·5 gm. POLYGLYCINE II
+ 5 m.l. SATURATED
AQUEOUS COPPER SULPHATE
SOLUTION, HEATED OPEN AT
72°C, FOR 24 hrs.

this reaction was a failure, but it does show that compounds of the Polyglycines can be prepared.

5. THE EFFECT OF SATURATED COPPER SULPHATE SOLUTION ON THE POLYGLYCINES.

Polyglycine I and II are both dyed by copper ions to a pale blue colour, on boiling with a saturated aqueous solution of copper sulphate. The X-ray diagram of the blue polymer shows no difference in position or intensity of the Polyglycine lines. No extra lines appeared for the copper. The Polyglycine I dyed polymer, showed Polyglycine I lines only. The dyed Polyglycine II photograph, showed that some change had occurred to Polyglycine I, the 3.45 A. line had appeared. Treatment of the blue polymers with 0.88-ammonia solution made the colour a little deeper. Treatment with 10% sodium hydroxide solution, changed the blue colour to pink, as in the biuret reaction.

0.5 gm portions of Polyglycine I and II were heated at 72°C, in the open, for 24 hours, with 5 ml. portions of saturated copper sulphate solution. The blue dyed polymers gave the X-ray lines for

Polyglycine I and Polyglycine II respectively. The conversion of Polyglycine II must therefore have been temperature dependent. The supernatant liquid contained no water insoluble polymer. The polymer obtained from the precipitation of a solution of Polyglycine I, in 70% aqueous zinc chloride solution, by alcohol; was also boiled with the saturated copper sulphate solution. This polymer was unchanged by the dyeing, and gave a Polyglycine I X-ray photograph. The polymer was dyed a deeper blue than in the previous two cases. The addtion of ammonia to the suspension left an even deeper blue residue, the supernatant solution was pink. The addition of sodium hydroxide solution dissolved the polymer leaving a clear pink solution.

The copper ions absorbed by the polymers could be removed by warming with dilute sulphuric acid, leaving the white polymer. Thus the absorption was reversible. It appeared that the polymer prepared from the zinc chloride solution had more available sites for absorption of copper ions, than did the other polymers.

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THE EXAMINATION OF THE POLYMERISATION OF GLYCINE IN TEN NORMAL HYDROCHLORIC ACID.

SUMMARY OF RESULTS.-

The products of the polymerisation of glycine in ten normal hydrochloric acid solution, produced two distinct solid layers on centrifuging. The bottom of the tube contained a gritty, dense polymer, and above this there was a layer of less dense, more gelatinous polymer. The less dense layer became increasingly more difficult to centrifuge down, as washing with water continued. Both the light and heavy fractions gave X-ray photographs which showed the presence of both Polyglycine I and Polyglycine II. The reaction times for the polymerisation were systematically varied, and the products from each reaction examined. It was found that Polyglycine II was the initial product of the reaction. This was then converted into Polyglycine I on more prolonged heating. Diketopiperazine was also found in the products.

DISCUSSION OF RESULTS.-

This work is the first observation of the intervention of Polyglycine II as an intermediate, in the fromation of Polyglycine I. The previous workers, see Experiments below, have stated that Polyglycine I only was formed. The work also shows that Polyglycine II is first formed in solution, and not in the solid state. The formation of Polyglycine I probably occurrs in the solid state by conversion of the Polyglycine II(solid), previously formed. This cannot easily be proved, and is certainly not proved in this work. The work also shows that the Polyglycine I prepared by this method contains about 5 to 10% of Polyglycine II, in order that the X-ray lines should appear. This discovery does not however invalidate the work done in experimental section 2., since this work compared the relative intensities of the X-ray lines before and after reactions.

The diketopiperazine which was found in the polymerisation products, was also present to the

extent of 5 to 10% of the product. The presence of this diketopiperazine has been confirmed by a chromatographic analysis of the reaction products. This product could only have been produced by the cyclisation of two molecules of glycine. It is not possible to say what part the diketopiperazine plays in the reaction.

EXPERIMENTS.

The polymerisayion of glycine in 10N. hydrochloric acid was first observed by Meggy[89]. He found that the highest yield of polymer was given by using 1 ml. of 10N. acid for every 5 gm. of glycine. This mixture was heated in a sealed tube at 140°C., for 18 hours. This gave Polyglycine I, reference Meggy and Sikorski[91]. This reaction was repeated many times, and it was observed each time, that in isolating the polymer some of the products settled out less readily than the remainder. The cooled tubes were opened, and the polymer washed out with hot distilled water. The suspension of polymer was then centrifuged down. The polymer settled out easily,

but formed two distinct layers. The bottom of the tube contained a dense gritty powder, and above this a more gelatinous, less dense layer. On washing with water this less dense residue, became less easily centrifuged down. Extensive washing with water, removing the hydrochloric acid, made this lighter residue even more difficult to centrifuge down completely. It was also noticed that on adding ethanol, to finish the washing, the gelatinous residue became aggegated into flocks. It was then possible to centrifuge the residue down completely.

Some of the lighter residue from the polymerisation was pipetted off from the centrifuged residue. This lighter suspension was divided into two parts. One part was washed with water only. The other was washed with first water, and then alcohol. Both residues were then dried over calcium chloride in vacuo. The X-ray photographs of the two residues were compared with that of the gritty polymer. The gritty polymer showed faint Polyglycine II lines, together with the main Polyglycine I lines. The two extracted residues, were also mixtures of Polyglycine I and II.

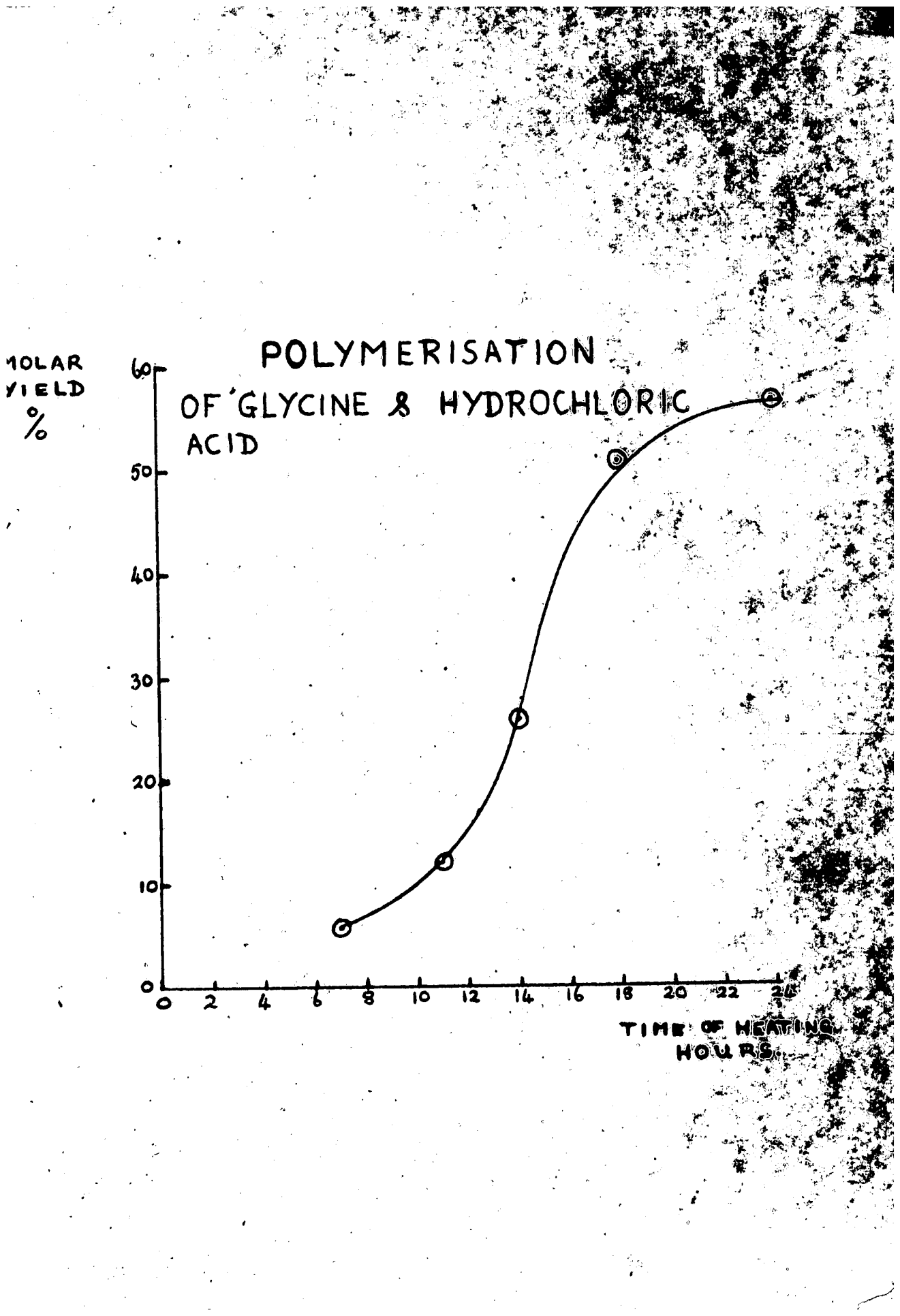
The Polyglycine II lines were relatively more intense in these samples. This showed that Polyglycine II was formed as an intermediate in the polymerisation.

It was decided to try to determine at what stage in the reaction, that the Polyglycine II was formed. 5 gm. portions of glycine and 1 ml. portions of 10N. hydrochloric acid, were sealed up in glass tubes. These tubes were placed in a glycerol bath at 140°C.. The solid glycine began to dissolve after two hours heating. After four hours heating the solid/liquid interface became "fuzzy." After five hours, the supernatant liquid became distinctly cloudy, the volume of solid glycine had distinctly decreased. After six hours, a white solid formed in the supernatant liquid. After seven hours, the first tube was removed from the heating bath, and rapidly cooled. The solid formed in the supernatant liquid was extracted with a little water, the extract centrifuged down, and the residue X-rayed. This photograph showed the lines for Polyglycine II only. The undissolved solid was also extracted with hot water. The product showed lines for Polyglycine II and other spotted lines.

These spotted lines were characteristic of the lines for glycine and for diketopiperazine. The products from this reaction have been investigated by chromatography, see experimental section 13.. This also showed that the product contained diketopiperazine. After nine to ten hours heating the undissolved solid slowly dissolved, or reacted, forming a cloud of polymer; solution was complete after eleven hours. On continued heating no further visible changes occurred. Tubes were removed at eleven, fourteen, and eighteen hours. The last tube was removed after 24 hours, the product gave the X-ray photograph of Polyglycine I, together with weak lines for Polyglycine II.

This experiment was repeated using electrical heating in place of the glycerol, to give better temperature control. The temperature was set to 140°C., using an air oven. The rate for reaction had increased slightly when compared with the previous experiment. The product obtained after seven hours heating was washed with water, until free from acid. It was then washed with ethanol, and dried in vacuo over calcium chloride. The

diffractometer trace showed some conversion of the Polyglycine II, first formed, into Polyglycine I, had occurred. The diffractometer trace, See Chart 3, at the end, showed the change far better than the photographs. The product from the tube withdrawn after eleven hours, was separated into the settled and unsettled residues. The unsettled residue X-ray photograph, showed a mixture of both forms of Polyglycine were present, but the Polyglycine I lines had increased in strength, with longer heating. The settled residue also showed a mixture of both Polyglycines, but the Polyglycine I line was not as intense as that for the unsettled residue. The yields were, 0.033 gm. for the unsettled residue, and 0.43 gm. for the settled residue, a total of 0.463 gms.. This compared with a value of 0.22 gm. after 7 hours heating. The tube heated for fourteen hours, was also similarly extracted into two portions. Again the unsettled residue showed an increase in the intensity of the 3.45 A. Polyglycine I line. The product still contained appreciable quantities of Polyglycine II. The yield of this residue was 0.375 gm.. The


POLYMERISATION
OF GLYCINE & HYDROCHLORIC ACID
MOLAR YIELD %
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME OF HEATING HOURS.

settled residue showed a mixture of both forms of Polyglycine, and again the 3,45 A. line was less intense than that shown for the settled residue. The yield was 0.64 gm.. The total yield was 1.015gm.. The tube withdrawn after eighteen hours heating, was also extracted to give two residues. The diffractometer traces show that the polymer has been almost entirely converted into Polyglycine I. The settled and unsettled residues did not show any line intensity differences, but the 3.45 A. line is now more intense than the 4.4 A. line, showing that conversion was almost complete. The yield was 2.00 gm.

The molar yield curve for the reaction is shown opposite, this assumes that the average degree of polymerisation of the Polyglycine is 10. This is the value given for stock Polyglycine I, prepared from glycine and hydrochloric acid. The method used to determine this value was that given by Meggy[89].

It was found to be impossible to extinguish the Polyglycine II lines completely, from the product Polyglycine I. If heating was continued for longer periods the yields were decreased, presumably due

to hydrolysis, and side reactions, and the Polyglycine II lines remained. it appears that all the Polyglycine I prepared by this method contains, from 5 to 10 percent of the Polyglycine II.

THE EXAMINATION OF THE POLYMERISATION OF GLYCINE IN GLYCEROL.

SUMMARY OF RESULTS.-

This polymerisation occurred by a similar mechanism to that in the previous section. Polyglycine II was obtained first of all, and this was converted into Polyglycine I on prolonged heating. The proportions of the two polymers present in the product depended on the volume of the liquid phase, e.g. the volume of glycerol added. The prolonged heating of the reaction mixture did not increase the concentration of Polyglycine I obtained. Where the volume of glycerol added was large, a greater proportion of Polyglycine II was obtained in the product. To obtain an appreciable proportion of Polyglycine I, a small volume of glycerol was required, 2 ml. per 5 gm. of glycine. The rate of reaction at this concentration was decreased. It appeared that the Polyglycine II was only converted to Polyglycine I, when its concentration in the glycerol solution reached a certain limit. This limit was not acheived in solutions containing more than 5 ml. of glycerol,

per 5 gm. of glycine. Similarly to the previous polymerisation, the presence of solid glycine at the reaction temperature was essential, and polymerisation appeared to occur in the liquid phase.

The polymerisation of glycine in mixtures of glycerol and water, gave lower yields of Polymer. The product in all cases was Polyglycine II only. Although the yields of Polyglycine II were low, the crystalline quality was very good, as was shown by the sharp X-ray lines. Increase of the concentration of water in the system, lowered the yield of polymer; until the use of a 25% glycerol/water solution, produced a yield of about 1% after 19 days heating at 140°C. An X-ray photograph of some of the wet reaction mixture showed lines for glycine and diketopiperazine, as well as very weak Polyglycine II lines. Thus as the activity of water in the system increased the reaction leading to polymer formation was repressed, and diketopiperazine was formed in preference to Polyglycine II.

The corresponding polymerisation of glycine in glycol, which gave diketopiperazine, also gave a small

quantity of hot water soluble polymer. This polymer showed the "leafing effect" on allowing to cool and crystallise. The X-ray photograph showed that this polymer was Polyglycine II. This is the first reported case of a low molecular weight polymer having a polyglycine II structure. This means that either the low molecular weight polymers crystallise in the Polyglycine II form only, or otherwise they exhibit isomorphism, like the higher polymers.

The brown fluorescent material produced during the polymerisation of glycine in glycerol has also been examined. Attempts were made to extract this brown colour from the solution remaining after the polymer had been separated. It was found that acetone did extract some of the brown colour from the solution. On evaporating off the acetone from this extract, a thick brown oil remained. The remaining colour could then be extracted by the addition of ether to the solution. The evaporation of the ether from this extract left a brown/green fluorescent oil. The acetone must have reacted with the compounds present in the solution, since extraction with ether was possible after addition of the acetone,

but had not been possible initially. Neither of the oils obtained could be made to crystallise. This work agreed with the observation of Maillard(1914)[86], that the brown material contained two components. Similarly, the solution from the preparation of diketopiperazine, by the polymerisation of glycine in glycol, contained a brown coloured product. This solution was treated with three times its volume of acetone, a chocolate brown precipitate slowly settled out. Most of the colour was removed from the solution on the formation of this precipitate. The X-ray photograph of the crystalline deposit, showed that it was neither glycine nor diketopiperazine, neither was it a mixture of these two compounds.

DISCUSSION OF RESULTS.-

The polymerisation of glycine in glycerol and glycol, was investigated as early as 1900 A.D.. In the case of glycine and glycerol, the products obtained were stated to be diketopiperazine and a horny Polyglycine. This work was carried out by Balbiano and Trasciatti(1900)[12], by heating the reaction mixture in a sealed tube at 170°C. The

product was said to be a "Polyglycine" since it was insoluble in the usual organic solvents, and could be hydrolysed to glycine. Maillard(1911,1914)[85,86], verified the above work, and showed that glycine dissolved in water, and diluted with glycerol, gave an 86% yield of diketopiperazine on heating at 170°C.. This work set out to determine which form of Polyglycine was obtained in this reaction. It has been shown that either form can be obtained. The volume of the glycerol, or glycerol/water mixture, added determined the proportions of each form of polymer obtained. A small volume of liquid phase also reduced the rate of reaction. Dilution of the glycerol solution, by water, resulted in a decrease of the yield of Polyglycine, and resulted at 75% dilution, in formation of diketopiperazine. This agreed with the work of Maillard except in the yield. The yield obtained was much lower. This could have been due to the lower reaction temperature of 140°C.. The increase of the activity of water in the system, resulted in the repression of polymer formation, and preferential formation of diketopiperazine.

The usual method for the preparation of

diketopiperazine, is from the polymerisation of glycine in glycol, in the ratio of 1 gm. of glycine to 5 ml.of glycol. Even this dilution by the glycol, has produced some water soluble Polyglycine II, but only soluble in hot water. This material has not been observed before.

The brown material which was obtained as a by product in the polymerisation of glycine in glycerol, or glycol, has not hitherto been examined. This has been because the material could not be separated, from the solution. This material has now been shown to be separable from both solutions. In the case of the glycerol solution. The product extracted by the ether, is probably not in the same form as it was originally present in solution. Reaction with the acetone has occurred, otherwise it would have been extractable by ether initially. Active methylene groups,or amine groups,are known to react with acetone. In the case of the glycol solution, a crystalline residue was obtained on extraction with acetone. The X-ray diagram for this is recorded, and shown to be a new by-product. It was decided that the further exploration of this material was outside the scope of this thesis.

EXPERIMENTS.-

1. Polymerisation in 100 percent glycerol.-

5 gm. of glycine were heated with 5 ml. of pure 100% glycerol, in a sealed Carius tube, at 140°C, in an air oven. The time of heating was 27 hours, the tube was removed from the oven and allowed to cool slowly. The tube was opened, and the product washed out with hot distilled water, this slurry was centrifuged down. The brown supernatant liquid was kept for examination. The solid was washed several times with water, and then washed once with alcohol. The residue was then dried in vacuo over calcium chloride. The residue was a very pale buff colour. The X-ray photograph showed that it was a mixture of Polyglycine I and II, with a greater proportion of Polyglycine II.

2. The effect of heating for a longer time.-

5 gm. portions of glycine and 5 ml. portions of glycerol were sealed up in tubes, and heated at 140°C. for the following times.

a. 24 hours.- the product was isolated as before. It showed some "leafing" on centrifuging down. The X-ray

photograph showed only lines for Polyglycine II. The molar yield assuming the polymer had a degree of polymerisation of 10, was 2.6% .

b. 48 hours.- The product was a very pale buff colour, the colour of the solution in the tube had become, much darker, than in the case above. The X-ray photograph of the product showed that it was a mixture of Polyglycine I and II. The Polyglycine II lines were more intense. The molar yield was 12,6 %.

c. 72 hours. The solution in the tube was almost black. The residue obtained was a pale buff colour. The X-ray pattern was the same as for specimen b. above. There was no increase of intensity of the Polyglycine I lines. The molar yield was 12.2 %.

d. 5 days.- The solution in the tube was again very dark. The residue was distinctly buff coloured. It again gave the X-ray diagram for a mixture of both Polyglycine I and II, andagain there was no increase in the intensity of the Polyglycine I lines. The molar yield was 11.8 %.

It was concluded that the reaction produced Polyglycine II as the initial product and that this this was converted to Polyglycine I. The yield of

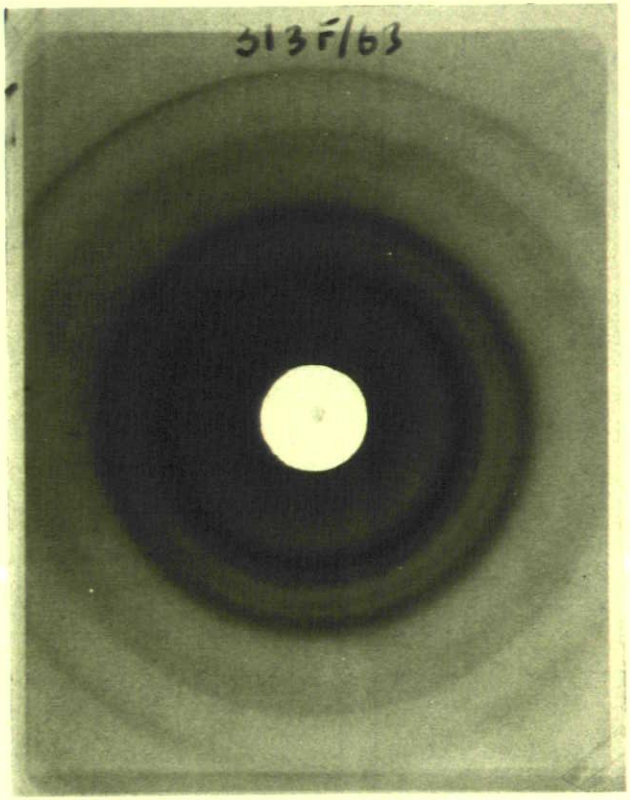
polymer increased for heating times of up to 48 hours. Polyglycine I was formed after 24 hours heating. The concentration did not increase appreciably after this first appearance. This may have been due to the effect of the competing side reactions, which tended to decrease the yield on longer heating, and darken the colour of the reaction mixture.

2. The effect of varying the ratio of the concentration of glycine to glycerol.

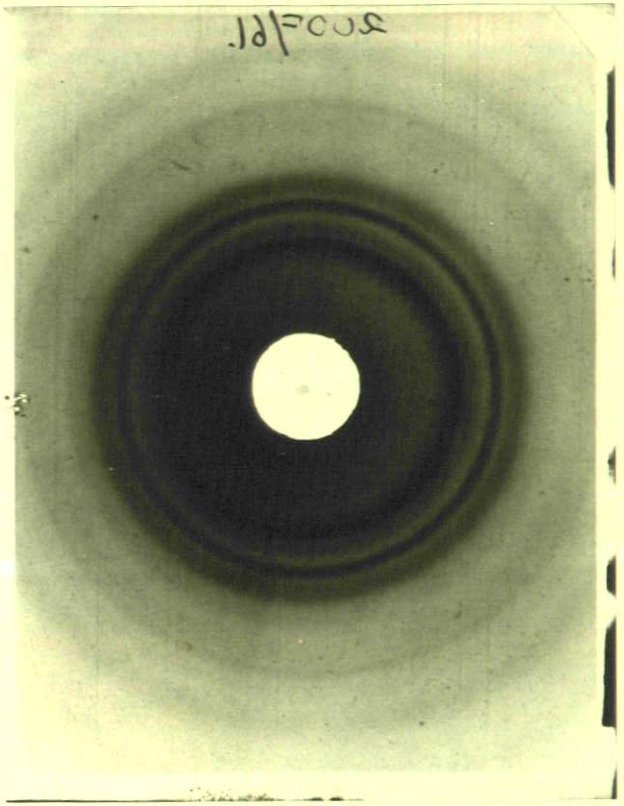
i) 5 gm. of glycine and 2 ml. of pure glycerol, were sealed up together, and heated at 140°C. for 4 days. The solution in the tube was a pale golden brown colour, and the separated product was almost white. The X-ray diagram of the product showed lines for both Polyglycine I and II. The intensity of the outer 3.45 A. line for Polyglycine I, appeared to be more intense than in the previous experiments. The molar yield was 8.9 %.

ii) 5 gm. of glycine and 1 ml. of glycerol were also heated sealed at 140°C. for 4 days. The glycerol was not able to wet the glycine completely. The dry glycine did not polymerise. The molar yield was 4.6 %. The X-ray diagram showed lines for Polyglycine I and II, with only a little Polyglycine I.

5 gm. GLYCINE + 10 ml 100% GLYCEROL
HEATED SEALED AT 160°C, FOR 72 hrs.



5 gm GLYCINE + 1 ml (100%) GLYCEROL
HEATED SEALED AT 160°C, FOR 4 DAYS.



5 gm GLYCINE + 2 ml (100%) GLYCEROL.
HEATED SEALED AT 160°C, FOR 4 DAYS.

POLYMERISATION OF GLYCINE IN GLYCEROL

111) 5 gm. of glycine and 10 ml. of glycerol was heated for 72 hours at 140°C. The solution was very discoloured, a dark brown. The polymer was a pale buff colour. The X-ray pattern showed a mixture of both Polyglycines, but the mixture contained a greater proportion of Polyglycine II. The molar yield was 15.9 %.

The minimum concentration of glycerol necessary, per 5 gm. of glycine, was 2 ml.. This ensured complete wetting of the glycine, and a reasonable yield of product. The molar yield was increased by altering the glycerol concentration to 10 ml. per 5 gm. of glycine, but the product did not contain the same proportions of the two polymers. Increase of the concentration of glycerol produced a greater proportion of Polyglycine II. The solution is much discoloured at this concentration. It is suggested that the Polyglycine II is attacked by a side reaction, and is not converted to Polyglycine I. The formation of Polyglycine II appeared to take place much more rapidly at this concentration. The minimum of glycerol is necessary to obtain an appreciable quantity of Polyglycine I in the product. The rate of reaction

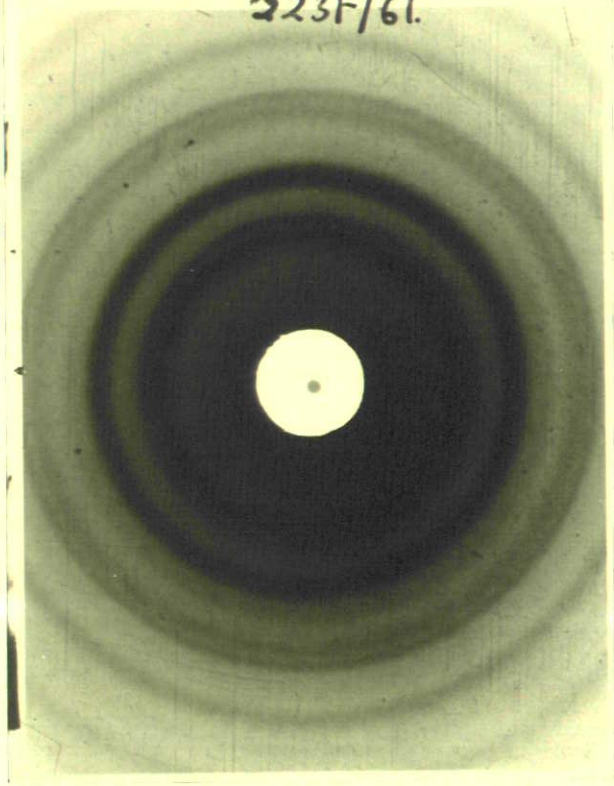
at this concentration is decreased, as is shown by the yield.

From the work in the preceeding section it seemed that the time of heating did not appreciably affect the proportions of the two polymers formed. The concentration of glycerol in the mixture had far greater effect. It is suggested that the formation initially of Polyglycine II takes place in the glycerol solution, this would account for the increase reaction rate on dilution with glycerol. Thus the rate of formation of Polyglycine II is proportional to the volume of the liquid phase. It may be that the Polyglycine I is only formed when the concentration of Polyglycine II in the glycerol, reaches a certain limit. This limit is not acheived in solutions containing more than 5 ml. of glycerol, per 5 gm. of glycine, owing to the rates of competing reactions.

4. Polymerisation of glycine in glycerol and water mixtures.

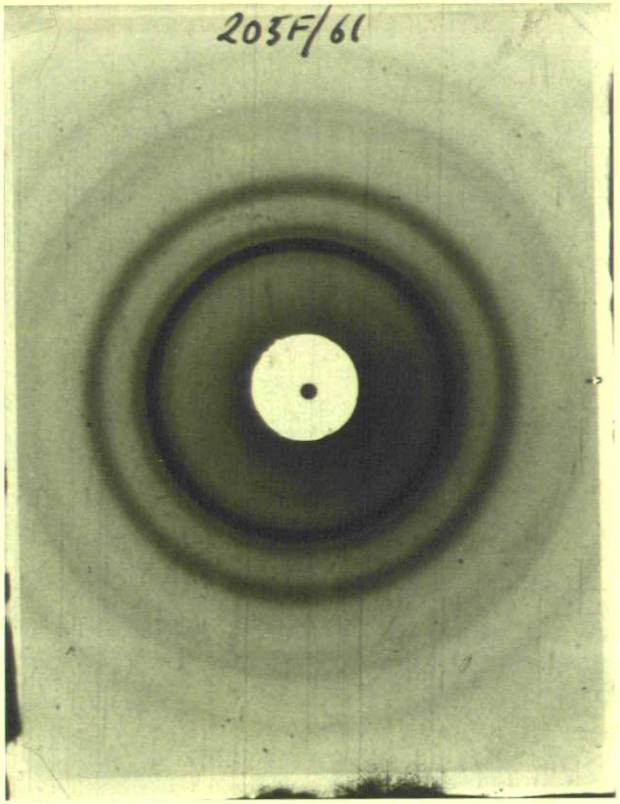
v. 4 gm. of glycine and 2 ml. of a 50% glycerol and water mixture were heated sealed at 140°C., for 4 days. The molar yield of polymer was 10.8 %. The product gave the X-ray pattern for Polyglycine II only. The product showed some "leafing" and the X-ray

223F/61.



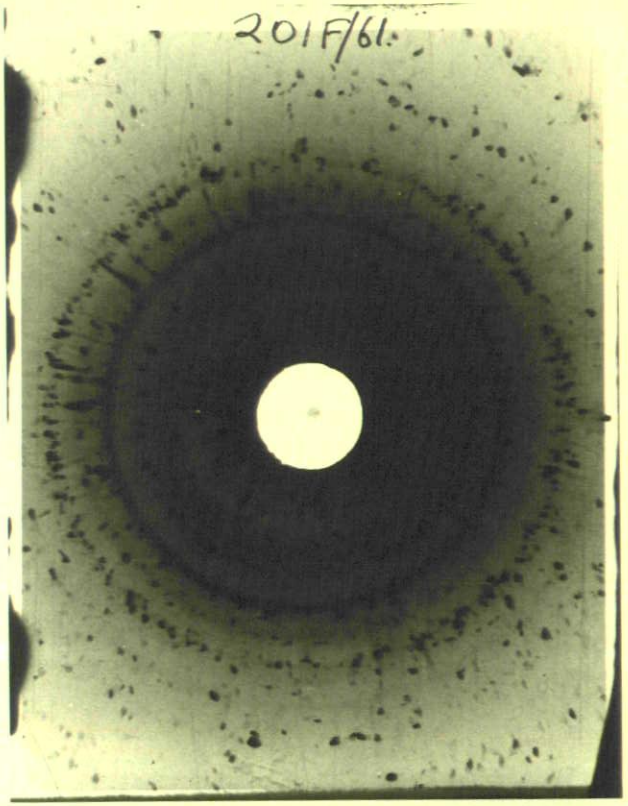
EXPERIMENT W. BULK PRODUCT.
POLYMERISATION IN 50% GLYCEROL.

203F/61



EXPERIMENT Z. BULK PRODUCT.
POLYMERISATION IN 25% GLYCEROL.

201F/61.



HOT WATER SOLUBLE RESIDUE
FROM EXPERIMENT Z.

POLYMERISATION OF GLYCINE IN GLYCEROL/WATER MIXTURES.

lines were quite sharp.

w. 10 gm. of glycine and 10 ml. of 50% glycerol and water mixthre, were heated sealed at 140°C. for 4 days. The molar yield of polymer was 10.6 %. The product was entirely Polyglycine II.

x. 10 gm. of glycine and 10 ml. of 50% glycerol and water mixture, were heated sealed at 140°C. for 6 days. The molar yield was 16.5 %. The reaction solution was very little discoloured, only a pale yellow colour. The polymer obtained was pure white. This seemed to be an excellent method for the preparation of Polyglycine II by direct polymerisation of glycine. The sample showed sharp X-ray lines.

y. 10 gm. of glycine and 10 ml. of 37½ % glycerol and water mixture, were heated sealed at 140°C., for 9 days. The molar yield of polymer was 8.3 % . The X-ray pattern showed lines for Polyglycine II only.

z. 10 gm. of glycine and 10 ml. of 25% glycerol and water mixture, were heated sealed at 140°C. for 7 days. Examination of the hot reaction mixture, showed that unlike the other preparations, there was no solid polymer. The hot solution was a pale

buff colour. On cooling a solid crystallised out, growing slowly from nuclei. The cold tube was opened and some of the wet residue was X-rayed. The photograph showed spotted rings for glycine and diketopiperazine. Most of this solid was soluble in hot water. The residue was washed with water and alcohol. This residue showed the X-ray pattern for Polyglycine II. The molar yield was however less than 1 %. This experiment was repeated and heating continued for 19 days. The polymer yield was still only about 1 %.

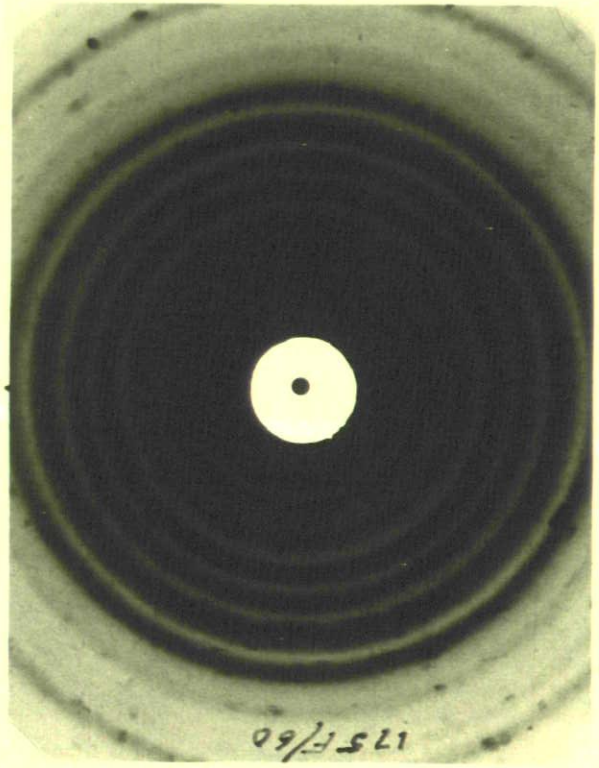
These reactions are of interest since they are the first recorded examples of the direct preparation of Polyglycine II, in a pure state. All other methods of preparation of Polyglycine II, have been carried out indirectly via Polyglycine I.

EXAMINATION OF THE BROWN SOLUTION OBTAINED DURING THE POLYMERISATION OF GLYCINE IN GLYCEROL AND DURING THE POLYMERISATION OF GLYCINE IN GLYCOL

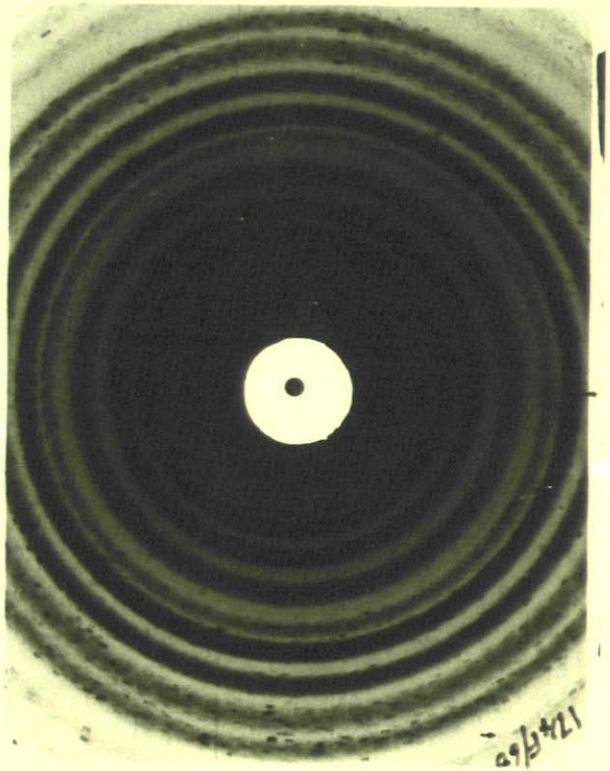
The brown supernatant liquid which was produced during the polymerisation of glycine in 100 % glycerol,

showed a green fluorescence. Attempts were made to extract this brown colour from the solution. Ether was first tried as an extraction medium, this did not extract the colour, and no solid remained on ~~extraction~~ the evaporation of the ether. It was found that a more hydroxylic solvent was necessary. If about three times its volume of acetone, was added to the brown solution, some of the colour was extracted into the acetone layer. The evaporation of this acetone extract, left a thick brown oily residue. This oil could not be made to crystallise. The solution remaining after the acetone extraction, was a pale brown colour. This remaining colour was then extracted by the addition of ether. The evaporation of the ethereal extract left a brown/green fluorescent oil. This oil occupied a greater volume than the first oily extract. This second oil could not be made to crystallise. The acetone must have reacted with compounds in the solution, and not acted merely as an extracting medium, for the solution to be ether extractable at the second attempt.

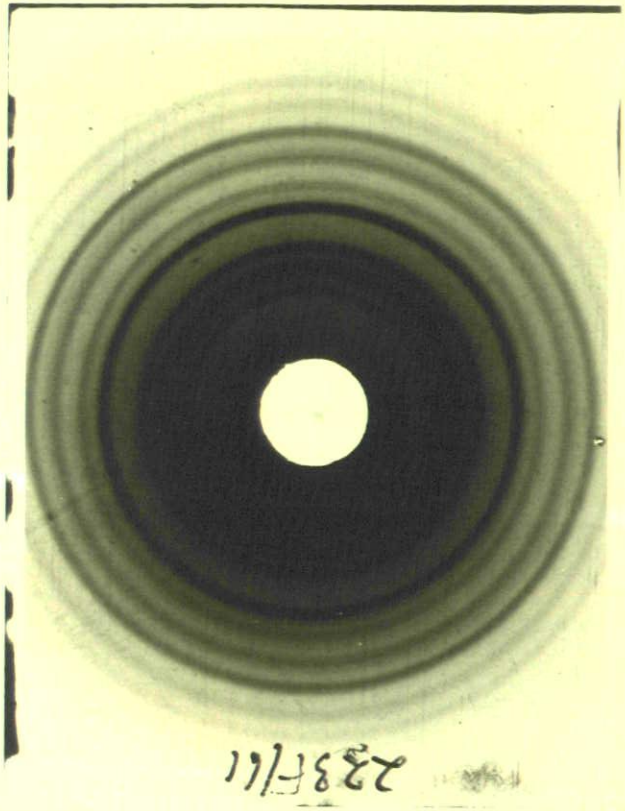
During the preparation of diketopiperazine



DIKETOPIPERAZINE



ANALAR.
GLYCINE



CHOCOLATE
PRECIPITATE
ex PREPARATION
OF DIKETOPIPERAZINE

SEC 3

Continued from page 15.

No.	ring diam. mms.	spacing A.	strength.
7	60.3	2.83	w
8	67.5	2.59	w
9	75.0	2.38	m
10	81.3	2.25	w
11	87.7	2.13	w

THE THREE STRONGEST X-RAY LINES FOR DIKETOPIPERAZINE.-

1	27.0	6.3	s
2	33.5	4.8	m
3	53.8	3.1	v.s.

THE THREE STRONGEST X-RAY LINES FOR GLYCINE.-

1	39.5	4.13	s
2	46.9	3.53	v.s.
3	72.0	2.45	m

THE X-RAY LINES FOR THE DRIED BROWN SLUDGE FOUND IN THE SPENT GLYCOL.

No.	diam. mms.	spacing A.	strength	material
1	27.0	6.3	w spotted	D.K.P.
2	38.9	4.15	v.s.	P.G. II.
3	53.0	3.11	w spotted	D.K.P.

---

from glycine and glycol, by the Schott, Larkin, Rockland, and Dunn[104] method, a similar brown material was produced. Treatment of this solution, with about three times its volume of acetone, caused the slow formation of a chocolate brown precipitate, on the walls of the separating funnel. The precipitate was crystalline, and its removal from the solution, removed almost all the brown colour. The precipitate was centrifuged down washed with ~~xxxxxx~~ acetone and dried in vacuo over calcium chloride. The solid was X-rayed, the photograph showed many lines. A comparison with similar photographs for glycine and diketopiperazine, showed that this material was distinctly different.

THE X-RAY LINES FOR THE BROWN ACETONE EXTRACT FROM THE PREPARATION OF DIKETOPIPERAZINE.-

No.	ring diam. mms.	spacing Angstroms	strength
1	28.5	5.55	w
2	32.6	4.90	v.s.
3	36.0	4.47	m.
4	39.2	4.15	s
5	43.9	3.72	v.s.
6	54.4	3.09	v.s.

Continued opposite.

If the spent glycol from the diketopiperazine preparation was allowed to stand for several days, a heavy brown sludge settled out. Some of this sludge was dried out in an air oven. The X-ray photograph of the material, showed the strong 4.15 A line of Polyglycine II, and two other weak spotted lines. The spotted lines were found to be the strongest lines for diketopiperazine at 6.3 A. and 3.1 A. by comparison of the measured photographs. It was found that during the working up of the diketopiperazine from the reaction solution, after it had been decolourised by boiling with charcoal in water and filtering hot, the filter paper always became clogged with a fine precipitate. This precipitate had quite a different appearance from the diketopiperazine. It was scraped off the filter paper and heated with a large excess of water. This removed any adhering diketopiperazine, which would have remained in solution. The solid residue was then centrifuged down, washed with water and alcohol and dried in vacuo over calcium chloride. This material showed the X-ray pattern of Polyglycine II only. This polymer could not have been Polyglycine II, since this is insoluble

in all solvents except strong aqueous salt solutions. This material had passed through a filter paper in the hot aqueous solution of the dione, during the working up. Glycine peptides having a degree of polymerisation of up to six are soluble in cold water. This product is only soluble in a hot solution. This suggests that it is a peptide of degree of polymerisation greater than 5 but less than 10, which crystallises in the Polyglycine II structure. Maillard[86] isolated a tetrapeptide and a hexapeptide from this preparation, which he found to be insoluble even in boiling water, but was soluble in an alkaline solution. This discovery of a low molecular weight peptide having a Polyglycine II type structure, agrees with that found in the products from the polymerisation of glycine in hydrochloric acid, see Exp. 2..

THE POLYMERISATION OF GLYCINE IN PHENOL.

SUMMARY OF RESULTS.-

The polymerisation followed the same general course as the polymerisations in hydrochloric acid and glycerol. Polyglycine I and Polyglycine II were obtained as products. Diketopiperazine was found in the solution produced by washing out the reaction mixture with water. The reaction rate was much slower, than in the previous polymerisations. The polymerisation of glycine in anhydrous phenol gave no solid insoluble product, but this may have been due to the short time of heating. Reactions were carried out in phenol and water mixtures. The reactions using the water rich phase of the phenol/water system did not produce any insoluble products. The phenol rich phase when used in the reaction did produce solid Polyglycine on prolonged heating. At equilibrium, at room temperature, the activity of water in the two phases is the same, but at 140°C. the system approaches more ideal behaviour. The critical solution temperature for the phenol water system, is 67°C. Thus the activity of water is much lower in the phenol rich phase at 140°C.,

than in the water rich phase. The Production of Polyglycine I only appeared to be exceptional. The more usual product was a Mixture of Polyglycine I and Polyglycine II, with Polyglycine I predominent.

The proportion of Polyglycine II in the product was dependent on the volume of the liquid phase, c.f. polymerisation in glycerol, where increase in the volume of the liquid phase increased the proportion of Polyglycine II.

The nature of the dark brown by-products obtained in the reaction solution were not investigated. A thick water insoluble tar was obtained, which would not crystallise. All the reaction tubes showed much internal pressure when opened, the nature of the reaction producing this pressure is not known. No ammonia, or methylamine odours could be detected on opening the tubes. The manner in which the tube contents effervesced on releasing the pressure suggested that carbon dioxide was dissolved in the phenolic solution. The raising of the reaction temperature to 160°C. resulted in the explosion of the tubes after one or two weeks heating.

The diketopiperazine found in the reaction products could only have been produced during the polymerisation reaction, or as a result of hydrolysis of the Polyglycine formed.

DISCUSSION OF THE RESULTS.-

This reaction was first investigated by Herzog and Krahn(1924)[67]. They heated glycine in cresol, in the ratiom of 1:10, at 190°C. in a sealed tube. The glycine was rapidly converted to diketopiperazine, giving a good yield. This work was intended as an extension of the earlier work, and as an examination of the effect of water on the glycine and phenol system. The results obtained showed very strong agreement with the results obtained with the previous polymerisation systems. This reinforces the general mechanism proposed for the direct polymerisation of glycine. The presence of diketopiperazine in the products also agrees with the previous results.

EXPERIMENTS.-

POLYMERISATION OF GLYCINE IN ANHYDROUS PHENOL.-

5 gm. of glycine and 5 ml. of phenol were heated in a sealed tube at 140°C. for 40 hours.

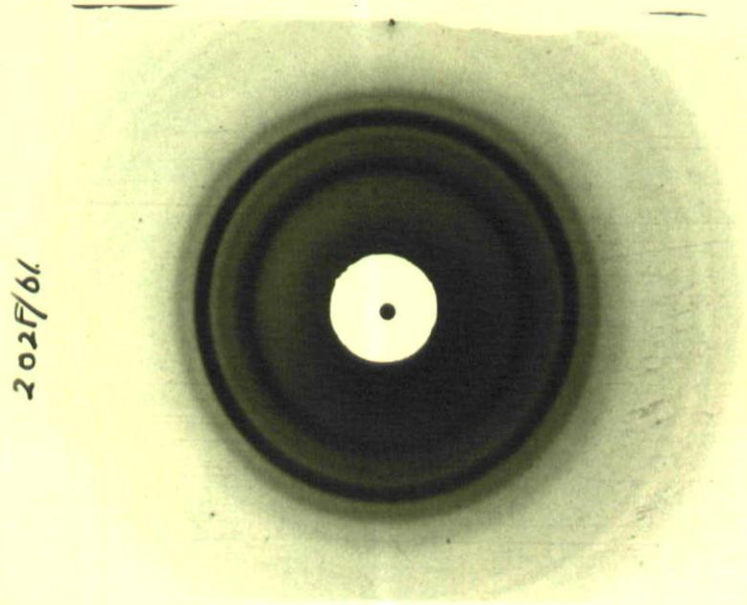
The tube was then allowed to cool, opened, and the contents washed out with distilled hot water. There was no water insoluble residue. The solution was then treated with twice its volume of ethanol, a heavy precipitate settled out. This material was extremely soluble in water. It was precipitated several times from aqueous solution by alcohol, to remove any phenol. The X-ray diagram showed only lines for glycine.

POLYMERISATION OF GLYCINE IN PHENOL AND WATER. -

A saturated solution of phenol in water was prepared at room temperature, i.e. a two phase system. Thus it was possible to pipettee off solutions of phenol in water which were either, rich in phenol, the lower phase, or rich in water, the upper phase.

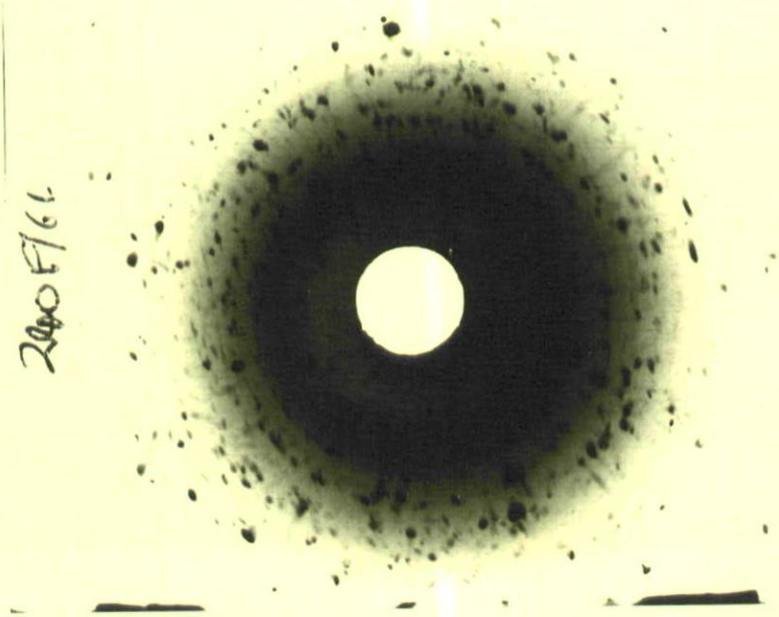
a. Polymerisation using the solution from the water rich phase.-

5 gm. of glycine and 10 ml. of the saturated solution of phenol in water, upper phase, were sealed up in a Carius tube. The tube was heated at 140°C. for 40 hours. The contents of the tube were



2021/61

POLYMERISATION OF
5 gm GLYCINE + 5 ml
PHENOL / WATER (LOWER PHASE)
HEATED SEALED AT 160°C
FOR 4 WEEKS



2040/61

HOT WATER INSOLUBLE
RESIDUE, DEPOSITED ON
STANDING, FROM SOLUTION

treated as above and again there was no water insoluble residue, ppecipitation by alcohol gave only glycine.

b. Polymerisation using the solution from the phenol rich phase. -

5 gm. of glycine and 5 ml. of the saturated solution of water in phenol, were heated sealed at 140°C. for 4 weeks. The contents of the tube became a brown colour, and there was much pressure on opening the tube. The solution appeared to contain much dissolved gas, which effervesced off on opening the tube. There was no odour of ammonia, and it was presumed that the gas was carbon dioxide. The contents of the tube were extracted with hot distilled water. A white insoluble residue remained undissolved. This residue was centifuged down, washed with water, and ethanol, and dried, in the usual way. The yield was 1.105 gm.(28%). The X-ray pattern for this product showed the lines for Polyglycine I only. The diffractometer trace chart 2, does show the presence of traces of PolyglycineII.

The aqueous solution from the above reaction was decanted off from the centrifuged solid and allowed

to stand for 2 weeks, a small quantity of white solid was deposited. The X-ray diagram of this solid was very spotted, no complet rings were visible, only parts of rings. Comparison with standard photographs for glycine and diketopiperazine, showed that material was diketopiperazine. The glycine used in the reaction was of A. R. quality, thus the diketopiperazine must have been formed during the reaction.

c. The effect of the time of heating

5 gm. portions of glycine, and 5 ml. portions of the upper phase of the phenol/water solution, were sealed up together, and heated at 135°C. for the following times.-

i) 5 weeks.- 0.22 gm. of insoluble Polyglycine II was obtained. The reaction took place much more slowly this time; no solid polymer was visible in the liquid for the first three weeks of heating. The yield was alsoconsiderably reduced.

ii) 7 weeks.- 0.7 gm. of polymer was obtained. This material showed strong X-ray lines for Polyglycine II, and weak lines for Polyglycine I , only the 3.45 A. line was at all distinct.

iii) 9 weeks.- 0.9 gm. of polymer was obtained. The X-ray diagram showed strong lines for Polyglycine II, and weak lines for Polyglycine I.

All the tubes showed much pressure of gas, on opening. The formation of polymer in the liquid in the tubes took place much more slowly in this case. The conversion of the Polyglycine II, first formed, into Polyglycine I, also took place much more slowly.

THE EFFECT OF VARYING THE GLYCINE TO PHENOL/WATER RATIO.-

a) 5 gm. of glycine and 1 ml. of solution of water in phenol, the upper phase, was heated sealed at 140°C. for 6 weeks. The yield of Polyglycine was 0.71 gms. The X-ray photograph showed that the material contained lines for both Polyglycine I and II of about equal intensities.

b) 5 gm. of glycine and 2 ml. of the solution, were heated sealed at 140°C. for 6 weeks. The yield was 0.63 gm.. The X-ray photograph showed that both forms of Polyglycine were present, but the Polyglycine I lines were less intense than those shown in a) above.

c) 5 gm. of glycine and 10 ml. of the solution, was heated sealed at 140°C. for 8 weeks. The yield of polymer was 0.44 gm.. The X-ray photograph showed lines for Polyglycine II only.

d) 5 gm. of glycine and 10 ml. of the solution of phenol in water, the lower phase, was heated sealed at 140°C. for 4 weeks. The tube was opened, showing some pressure, and the solid washed out with hot water. A very small quantity of mixed brown and white solids remained insoluble in the water. The mixed solids were centrifuged down and the aqueous solution decanted off. The brown solid was soluble in ethanol, it was probably an oxidation product of the phenol. The white solid remained insoluble. The quantity of the solid was too small to obtain an X-ray photograph.

e) 5 gm. of glycine and 5 ml. of the lower phase solution was heated sealed at 140°C. for five weeks. No solid water insoluble product was obtained. A second sample was heated for 9 weeks. This also produced no insoluble residue. The solutions from both experiments deposited glycine only on standing, and treating with alcohol.

These experiments showed that it was possible to obtain both pure Polyglycine I and Polyglycine II, simply by varying the ratio of the concentration of

glycine to water in phenol solution. Polymerisation of glycine in the phenol in water solution, was much less ready. Attempts were made to increase the reaction rate by carrying out the polymerisation at 160°C.. This was not successful, the tubes usually exploded after several days heating. Polyglycine I was obtained only if the proportions were 5 gm. of glycine to 1 - 5 ml. of water in phenol solution, at temperatures above 140°C.

THE POLYMERISATION OF GLYCINE HEATED ALONE.

SUMMARY OF RESULTS.-

Glycine was heated in a sealed tube at 170°C.. The results were not reproducible, this was probably due to variations in the moisture content of the glycine used. One sample gave a low yield of polymer, which proved to be Polyglycine I. The polymer was discoloured a pale buff colour. The polymer also showed the "leafing effect," this had been previously thought to be a characteristic of Polyglycine II samples. The X-ray lines of the material were quite sharp, but the diffractometer trace showed that the material was only of little better crystallinity. It was found that if the glycine had been previously dried over calcium chloride in a vacuum desiccator, no polymer was obtained on heating sealed. Glycine taken from the storage bottle always gave Polyglycine I only on heating, the yield was always very low. The heating of glycine in water at 140°C. for a week gave no polymer. When the same mixture was heated sealed at 160°C., the product was always the mixture of Polyglycine I and Polyglycine II. This material

showed a division of easily and less easily, centrifuged material. The solution was also shown to contain diketopiperazine.

DISCUSSION OF RESULTS.-

The results showed that the glycine would not polymerise unless there were traces of moisture present, thus the water acted as an initiator. This was in agreement with the results obtained from the polymerisation of glycine in hydrochloric acid, where the polymer first appeared in the liquid phase. The merely damp glycine, gave a product of Polyglycine I only. The glycine which had been heated with water, gave the product containing Polyglycine I and Polyglycine II. This agreed with the idea that no Polyglycine I was formed unless the concentration of the Polyglycine II in the solution reached a certain level. This reaction has also followed the proposed mechanism for polymerisation, but in the case of the damp glycine the rate of conversion of Polyglycine II into Polyglycine I has been increased because the volume of the liquid phase was very small.

EXPERIMENTS.-

Curtius and Benrath[40] showed that when glycine was heated alone at a high temperature it polymerised, giving peptides of glycine and diketopiperazine as the products. They assumed that the peptides were mainly the pentapeptide. This work was followed up by Abderhalden and Komm[2](1924). They heated glycine in a sealed tube at 160°C. and obtained Polyglycine and diketopiperazine. This work has been repeated in order to determine the structure of the Polyglycine obtained.

a. 5 gm. of stock glycine (A.R.), was heated sealed at 180°C. for two weeks . The tube contained a dark brown sticky mass, this was washed out with hot water. A buff coloured water insoluble polymer was obtained. The yield was 0.32 gm.. The product showed the "leafing effect", but the X-ray photograph showed that it was Polyglycine I only. The diffractometer chart trace, see chart 3, also showed that the residue was pure Polyglycine I. The polymer was of comparable purity to that obtained from glycine and water in phenol, see chart 3.

b. A similar portion of glycine was heated sealed at the same time as a) above, and for the same length of time. The contents of this tube were a dark brown liquid, with a high viscosity. On dilution with water no insoluble product remained.

The tubes a) and b) showed only a little internal pressure on opening.

c. 5 gm. of stock glycine was heated sealed at 150°C. for 4 days. The product was pure Polyglycine I.

d. 5 gm. of stock glycine was dried over calcium chloride in vacuo. This was placed in a sealed tube and heated at 150°C., simultaneously with c) above. The reaction was so slow that heating was continued for 3 weeks. There was no water insoluble residue.

e. 20 gm. of glycine and 4 ml. of water was heated at 140°C. for one week. There was no reaction.

f. 20 gm. of glycine and 4 ml. of water was heated sealed at 160°C. for 48 hours. The water insoluble residue could be separated into two portions on centrifuging. The less easily centrifuged down portion was decanted off and centrifuged down separately. This product was shown to be Polyglycine II. from its diffractometer trace, see chart 3.

The more easily centrifuged residue, gave an X-ray diffractometer trace showing lines for both Polyglycine I and Polyglycine II, the concentration of the Polyglycine I was greater than the Polyglycine II. The supernatant solution from the centrifuging down was also examined. A chromatogram (butanol, pyridine, water, 1:1:1. upper phase), showed that the solution contained diketopiperazine. This must have been formed in the course of the formation of the Polyglycine I.

THE POLYMERISATION OF BENZOYL GLYCINE AND GLYCINE, AND PHTHALYL GLYCINE AND GLYCINE.

SUMMARY OF THE RESULTS.-

The work of Curtius and Benrath(1904)[40] showed that it was possible to polymerise glycine and the ethyl ester of hippuric acid. They did not succeed in polymerising glycine and hippuric acid, benzoyl glycine. The polymer which they obtained was claimed to be the benzoyl derivative of pentaglycylglycine, on the basis of an elementary analysis. This method of analysis is unreliable in this instance. The figures shown below give the percentages of carbon and nitrogen in the benzoylated derivatives of glycine hexapeptide, and the decapeptide.

Benzoyl- G_6. Carbon(19 atoms)49%, Nitrogen(6)18.9%.
Benzoyl- G_{10}. ,, (27 ,,)47%, ,, (10)20.2%.

Thus the percentages of carbon and nitrogen vary very little for the addition of four glycine units.

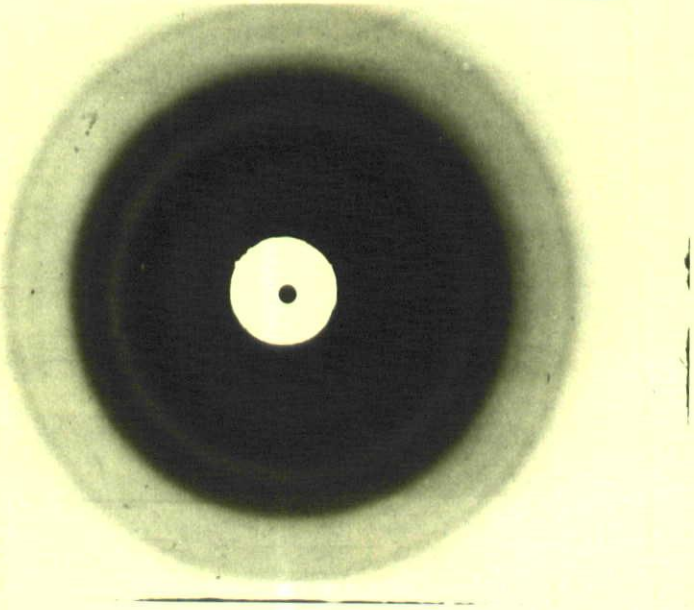
It has now been found possible to polymerise glycine and benzoyl glycine. The polymeric product was soluble in hot water, but crystallised out on cooling. Thus it was a low molecular weight peptide, and not a Polyglycine. The product was homogeneous

since it could be crystallised without change. The X-ray photograph showed the intense 4.15 A. line of Polyglycine II, and the strongest line of benzoyl glycine, and only one other line. None of the other strong lines of benzoyl glycine were present, showing that the material was not a mixture of benzoyl glycine and Polyglycine II. The product appears to be the benzoyl derivative of Polyglycine II. This has been confirmed by studying the X-ray diffractometer chart traces for the materials. The maximum possible number average degree of polymerisation was determined. The value obtained was 5.8, thus it did not quite correspond to the pentabenzoylglycine of Curtius, this assumed that the product was completely benzoylated. The relatively easy solubility of the material, considering the degree of polymerisation, can be explained. The benzoyl derivative would not have the zwitter ion structure, and would therefore be more soluble.

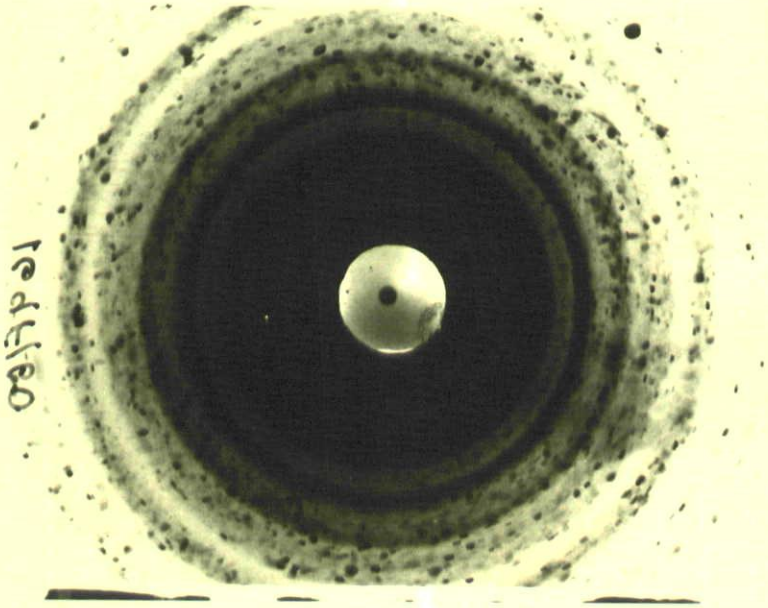
The polymerisations carried out in sealed tubes, were such that the proportions of glycine and water would have given a polymeric product in the presence of an acid catalyst. In the presence of the benzoyl

glycine the solubility of the glycine was increased, and no solid phase was present at the reaction temperature, and no polymer was formed. When the molar ratio of glycine to benzoyl glycine was increased, and heating prolonged, some Polyglycine II was obtained, together with a trace of Polyglycine I. This reaction again supported the idea that Polyglycine II was the initial product of polymerisation, and that this was only converted to Polyglycine I, when a limiting concentration of Polyglycine II was reached.

Similar results were obtained when glycine and phthalyl glycine were heated in a sealed tube. The natural acidity of the benzoyl and phthalyl derivatives, seemed to aid the the polymerisation of the glycine, but the increase in the solubility of the glycine, restricted the polymer formed to Polyglycine II, only. The hydrolysis of the benzoyl and phthalyl glycines was not very rapid even at 180°C. This was not expected from Thermodynamics.



MELT POLYMERISATION OF
EQUIMOLAR PROPORTIONS OF
BENZOYL GLYCINE & GLYCINE



BENZOYL GLYCINE

EXPERIMENTS.-

a. Polymerisation by heating in the open air.-

10 gm. of glycine and 10.5 gm. of benzoyl glycine(molar ratios 2.3 to 1) were ground up together in a mortar. The mixture was heated in the open at 190°C. when it became molten. It was maintained at this temperature for about 5 minutes, with constant stirring. The mixture became a very stiff paste. This was then allowed to cool. The cold solid was broken up and boiled with 500 ml. of water, and one gram of animal charcoal, and filtered hot. This gave a brown product, a second recrystallisation from hot water and charcoal gave a buff powder. This was dried in vacuo over calcium chloride. The yield was 0.8 gm.. The melting point of the product was between 280° and 290°C., that of the Curtius product was 280° to 285°C., with decomposition. The X-ray photograph of the material, showed lines at.-

No.	ring diam. mms.	strength	spacing A.
1	19.8	v.s.	4.15
2	26.5	m.	3.20
3	28.2	m.	3.00

The line no. 1 corresponded exactly to the 4.15 A. line for Polyglycine II. The other Polyglycine II

line, is at 3.1 A. this lies between the lines 2 and 3, which could have been masking it. Line 2 coincided with a strong line on the X-ray diagram for benzoyl glycine. The other strong lines of the benzoyl glycine diagram did not appear. The product was most certainly not just Polyglycine II. It was homogeneous since it could be recrystallised from water without change. The degree of polymerisation was determined by titration against caustic soda, after the addition of formol, according to the method of Meggy(1958)[88]. This measured the maximum possible value of the degree of polymerisation, and gave a value of 5.5, this assumed that the material was completely benzoylated.

The filtrate from the reaction was allowed to stand, this deposited a further crop of product. This appeared to be identical according to the X-ray photograph with the first crop of material. An X-ray diffractometer trace, see chart 1, showed that there was some difference. It also showed that the first produced material showed low angle spacings These diffractometer traces are further dealt with in section 17..

b. Polymerisation by heating in a sealed tube.-

i) 4.5 gm. of glycine and 1.79 gm. of benzoyl glycine were sealed up in a tube together with 1 ml. of water. The tube was heated at 140°C. for eleven hours. All the solid dissolved. An extremely small quantity of water insoluble residue was obtained, on washing out the tube with hot water.

ii) Similar proportions of glycine and benzoyl glycine (mole ratio 6:1) were sealed up with 1 ml. of water and heated for 40 hours at 140°C. The yield of water insoluble product was considerably increased, 0.44 gm. being obtained. The product was a pure white powder. This gave the X-ray diagram for Polyglycine II and a faint 3.45 A. line for Polyglycine I.

iii) Equimolecular quantities of glycine and benzoyl glycine, 5.9 gm. of the mixture, were dissolved in the minimum of water at the boil, this required 1 ml.. The same proportions of solid mixture and water were placed in a tube, sealed up and heated at 180°C. for 1.5 hours. All the solid dissolved at 180°C. The reaction tube contained rose coloured crystals and solution.

These crystals gave the X-ray photograph for benzoyl glycine only.

The polymers obtained by this method were distinctly of the Polyglycine II form no lines due to benzoyl groups could be detected, in the X-ray diagram. The photogaphs showed lines for Polyglycine II and I only.

Polymerisation of phthalyl glycine and glycine.- Heating in a sealed tube.-

8.2 gm. of phthalyl glycine and 3.0 gm. of glycine, equimolecular quantities, were sealed up in a tube together with 2 ml. of water. The tube was heated at 180°C. for 1.5 hours. All the solid had dissolved in the hot solution, which remained colourless. Crystals were deposited on cooling. The X-ray photograph of these crystals showed that they were phthalyl glycine only.

2.05 gm. of phthalyl glycine and 4.5 gm. of glycine, and1 ml. of water were heated in a sealed tube at 140°C. for 11 hours. about 25 mgm. of water insoluble polymer was obtained. This gave the X-ray photograph of Polyglycine II only. The molar ratiox of the reactants was 6 mole of glycine to 1 mole of phthalyl glycine.

This reaction seemed to proceed on exactly similar lines to the benzoyl glycine and glycine polymerisation. In both cases much longer heating would be required to produce significant quantities of polymer. The presence of the two derivatives of glycine in the reaction mixtures does at least initiate slow polymerisation reactions. The rate of reaction is increased compared with the polymerisation of glycine in water, thus the compounds of glycine can act as initiators and or catalysts for the reaction. The products are not however pure peptides, but must contain some benzoyl or phthalyl terminated molecular chains.

THE POLYMERISATION OF DIKETOPIPERAZINE IN WATER.

SUMMERY OF RESULTS.-

The polymerisation of diketopiperazine has been shown to proceed by the same general method as has been proposed for the other polymerisation methods. Polyglycine II has been found in the products, when the mixture has only been heated for a short time, 4 hours. When heating was prolonged, at 170°C. for 6 hours, pure Polyglycine I was obtained as the product. The usual effect was shown on increasing the ratio of the concentration of water to diketopiperazine in the reaction mixture. The increase of this ratio, decreased the reaction rate, and increased the concentration of PolyglycineII in the reaction mixture. A comparison of the diffractometer traces of the reaction products with that for pure diketopiperazine showed a striking phenomenon. In all cases only some of the strong diketopiperazine lines appeared in the reaction product traces. Some of the diketopiperazine lines were completely absent. This was not a case where the intensity of the trace due to diketopiperazine as a whole had been reduced, but one where only selective peaks were present.

DISCUSSION OF RESULTS.-

The polymerisation of diketopiperazine in water was first reported by Polyakova and Vereschagin(1949)[98]. They showed that if the reactants were heated at 170°C. under 200 to 400 atm. pressure, about 25% of the diketopiperazine was converted to Polyglycine. Meggy(1953)[88] showed that the use of high pressures was not necessary, and carried out polymerisations at temperatures between 60°C. and 180°C., by heating in Carius tubes. He showed that the yield of Polyglycine was dependent on the time of heating and the ratio of water to diketopiperazine. The presence of water was found to be essential for reaction to occurr. The optimum proportions were found to be 0.5 to 1 part of water, to one part of diketopiperazine. No polymer was formed if this ratio exceeded 3:1 . The formation of polymer was rapid, the maximum yield was acheived in 3 to 6 hours at 180°C. He suggested that an approximate equilibrium was rapidly established between the solid polymer and the diketopiperazine in solution, and that polymer formation ceased at this point. The balance of this reaction was continuously disturbed by hydrolysis

and decomposition reactions. He also calculated an approximate value for ΔF, for the reaction.- solid diketopiperazine -> solid polymer, at 180°C.. This gave a value for $\Delta F = -260$ cal.(approx.). Thus the polymer is the stable phase, into which the diketopiperazine is converted at 180°C.. The margin of stability is small, but he states that at 140°C. polymer is still the stable solid phase. The second paper Ref. 89, showed that ΔF changed very little with temperature. Thus in the presence of an aqueous phase, diketopiperazine would be expected to be unstable with respect to glycine and Polyglycine at all temperatures.

The reaction has been examined in order to determine whether it followed the same course as the other polymerisations. In order to determine whether Polyglycine II was first formed the reaction was carried out at 160°C. This meant that the reaction was complete in 8 to 12 hours. The reaction did follow the general pattern, Polyglycine II was formed first. But the method of formation from diketopiperazine may be different from previous theories of the reaction mechanism.

The diffractometer traces have shown, by comparison with diketopiperazine traces, that in all the polymerisations only some of the diketopiperazine lines appeared. Only certain of the strong lines have been reduced in intensity, see Chart 5. This seemed to indicate that the Polyglycine II is formed in the solid state at the expense of solid diketopiperazine. The Polyglycine II being formed by a rearrangement of the diketopiperazine lattice along one at least, of its crystallographic axes. If this reaction occurred in solution it would be expected that some diketopiperazine would have remained in its original crystalline state, this is not observed in the traces. These observations are entirely new, and may have a considerable bearing on the elucidation of the structure of Polyglycine II, and the author regrets that they were not obtained at an earlier stage in the investigation, so that more conclusions could have been reached.

The reaction was also carried out at 170°C., so that the reaction could run to completion. The product was pure Polyglycine I. This was the best method for the preparation of pure Polyglycine I,

which of those which have been investigated in this thesis. The diffractometer trace, see Chart 8., showed that although the main lines were quite sharp, the remaining lines were present as broad bands of lines. This material was produced specifically for a study of the strudture of Polyglycine I, this is dealt with in the section on X-ray diffraction of the Polyglycines, see Sec. 16..

EXPERIMENTS.-

The experiments were carried out by heating in sealed Carius tubes, in an air oven. The hot tubes were withdrawn at the end of the reaction, allowed to cool, opened, and the contents extracted with hot water. The cold water insoluble residue, was washed three times with cold water, and once with ethanol, and centrifuged down. The residue was dried in vacuo over calcium chloride.

a. 10 gm. of diketopiperazine and 5 ml. of water, were heated sealed at 170°C. for 6 hours. The yield of polymer was 6.08 gm. of pure PolyglycineI . The diffractometer trace , see Chart 8, showed no trace of Polyglycine II lines. The crystalline quality of this sample was not high, but it was the best obtainable.

b. 5 gm. of diketopiperazine and 5 ml. of water, was heated sealed at 160°C., for four hours. The diffractometer trace, see Chart 5., of the product, showed a very strong line at 4.15 A. for Polyglycine II. Peaks also occurred at 4.4 A.(m), and 3.39 A.(s), these were characteristic of Polyglycine I. The remaining peaks were all due to diketopiperazine.

c. 5 gm. of diketopiperazine and 2.5 ml. of water, was heated for 5 hours at 160°C. The diffractometer trace showed that the previously strong 4.15 A. line had now become quite weak, and the Polyglycine I lines predominant. The diketopiperazine lines were also of reduced intensity.

d. 10 gm. of diketopiperazine and 7 ml. of water was heated at 160°C. for 6 hours. Again the Polyglycine I lines were predominant over the Polyglycine II lines. The diketopiperazine lines were still present. The effect of an extra one hour's heating had been completely offset by the increase in the proportion of water present as compared with reaction c. above.

THE POLYMERISATION OF GLYCINE IN PHOSPHORIC ACID.

SUMMARY OF RESULTS.-

This method of polymerisation is the only reliable method for the polymerisation of glycine at normal pressures. It has been shown that both forms of Polyglycine can be obtained by heating in the open. This method of heating the reactants in a melt, in the open, is the best method for the production of low molecular weight forms of Polyglycine I and II . Neither of these low molecular weight forms have been observed before. It seemed that heating in the open or in a sealed tube produced polymer containing material of a wide range of degrees of polymerisation, it may also be possible to obtain high molecular weight products as well. The polymerisation followed the general mechanism, Polyglcine II being formed first, and then Polyglycine I. The diffractometer trace for one product, showed the presence of partially converted diketopiperazine, in the product, this has been confirmed by chromatography. This material like that obtained in the case of the polymerisation of diketopiperazine, see Sec. 7., showed only some of the strong X-ray peaks expected.

A peculiar product was also obtained, which was hot water soluble, and showed long, low Bragg angle, X-ray diffraction spacings. This material is probably a low molecular weight polymer.

The hot water soluble form of Polyglycine II could be recrystallised from boiling water. The crystals obtained were very thin hexagonal leaflets, of microcrystal dimensions, only visible under the electron microscope. The hot water soluble form of Polyglycine I was also examined under the electron microscope but showed no distinct crystal habit.

DISCUSSION OF RESULTS.-

This method of polymerisation is only the second recorded method for the polymerisation of glycine at atmospheric pressure. The other method was that recorded by Meggy(1956)[89], for the polymerisation of glycine in hydrochloric acid. This new method is superior as regards the yields obtained, and the variety of peptides obtained. This method is the best method for the preparation of low molecular weight forms of both Polyglycines. These low

molecular weight peptides have not been observed before. This method of polymerisation needs further investigation, it should be easily possible to obtain better crystals of both forms of Polyglycine, of low molecular weight. It may even be possible to obtain single crystal specimens in this way. The well defined hexagonal microcrystals of hot water soluble Polyglycine II, see Sec. 14,, appeared to be the same material as was obtained by Meggy and Sikorski(1956)[91], in a chance preparation which they considered was Polyglycine II, of high molecular weight. No distinct crystal habit was shown by the Polyglycine I, low molecular weight, under the electron microscope.

The polymerisation can be controlled and can be made to give a wider range of peptides, of wider degrees of polymerisation, and in greater quantities, than any other method. This method would be ideal for the study of the mechanism of the direct polymerisation of glycine. The peculiar low-molecular weight product showing low Bragg angle X-ray reflections, See Sec. 17., should also be further investigated.

The polymerisation followed the general mechanism proposed for the other direct polymerisations of glycine. Polyglycine II was formed first and this was converted into Polyglycine I on more prolonged heating. Diketopiperazine which has been partially converted into Polyglycine II, has been found in one of the products, See Chart 9. showing the diffractometer trace. The presence of diketopiperazine in the product has also been shown by chromatography. This partially converted diketopiperazine trace ~~xx~~ can be compared with similar traces obtained in Section 7.. In common with these traces, only some of the diketopiperazine lines appear. This suggests again that at least some of the glycine is converted first to diketopiperazine and then to Polyglycine II. It is not possible to say at this stage whether this reaction is intermediate in the main polymerisation, or is a competing side reaction.

The polymerisation was also carried out in sealed Carius tubes. The products were always the same mixture of polymers as obtained by heating in the open. It was however noticed that a little of the product was insoluble in saturated aqueous calcium

chloride solution. This suggested that some higher molecular weight products, than the Polyglycines, were also present in these products. This would be a very interesting and important discovery if it proved to be true.

EXPERIMENTS.-

a. Polymerisation by heating in theopen.

Preliminary experiments were carried out by heating glycine in 85% phosphoric acid in the open air. The boiling point of the phosphoric acid alone was 150°C.. Addition of even small quantities of glycine raised the boiling point to 165°C. If the heating was carefully controlled so that the temperature remained at 165°C., it was possible to boil off much of the water formed on polymerisation. This could be done without the solution becoming black and charred. The optimum proportions were found to be 40 gms. of glycine to 10 ml. of 85% phosphoric acid. The acid was heated to near its boiling point and the glycine added slowly. When the addition of glycine was complete, heating was continued with constant stirring. The mixture of

undissolved glycine and solution became first of all, a pale brown colour. This colour slowly deepened. A little of the mass was extracted and allowed to cool. It set to a hard yellow fluorescent mass, containing crystals of glycine, all of this mass was soluble in water. Heating was continued until the mass became suddenly viscous. The mass was then allowed to cool somewhat, and then boiled and stirred with hot water. Otherwise on complete cooling it set to a hard "glass." The solution was allowed to cool and the solid polymer centrifuged down. The polymer was washed with water and alcohol, and dried in vacuo. 7.3 gm.(17%) of greyish white polymer was obtained. The X-ray diffraction trace showed that this material had a Polyglycine II structure. It was found that about 10% of this material was soluble in near boiling water. The insoluble residue was filtered off, and the filtrate allowed to cool. A crystalline deposit which showed pronounced "leafing" was obtained from the filtrate. The X-ray diffractometer trace of this material showed that it also showed a Polyglycine II type structure.

This hot water soluble, and therefore low

molecular weight, Polyglycine II was recrystallised by redissolving in hot water. The recrystallised specimen showed more pronounced "leafing". The electron microscope showed that this material consisted of thin hexagonal leaflets showing more pronounced growth steps and better general definition than the material obtained from glycine and hydrochloric acid.

A repeat of the above experiment showed that if heating was continued, past the point at which the solution became viscous, the mass became very dark, almost black. The insoluble product was a distinct grey colour, and the X-ray photograph showed that both Polyglycine I and II were present. This crude product was extracted by boiling with water. The filtrate from the extraction, deposited a pale buff "leafing" material on cooling. This was found to be Polyglycine II only. This material was centrifuged down and the mother liquor treated with an equal volume of ethanol. A deposit was obtained from this solution on standing. This residue gave a characteristic Polyglycine I X-ray photograph. This is the first recorded occurrence of a low molecular weight , cold water soluble, Polyglycine I.

It had been previously thought that the lower molecular weight polymers crystallised only in the Polyglycine II form. This precipitation by ethanol of Polyglycine I from solution, agrees with the results obtained by precipitating solutions of Polyglycine in saturated aqueous zinc chloride solution, by alcohol, and obtaining ill defined Polyglycine I. It seemed that the precipitant determined the form of Polyglycine obtained. The Polyglycine I obtained in this case was crystallographically well defined.

40 gm. of glycine was added slowly to 5 ml. of near boiling phosphoric acid, heating was continued until the mass started to thicken, heating was stopped and water added immediately, about 150 ml.. The hot solution was filtered, the filtrate deposited Polyglycine II on cooling. The solid residue was treated with another 150 ml. of water and boiled. The hot solution was again filtered. A second hot waster soluble residue was obtained on cooling. The X-ray diffractometer trace, see Chart 10., of this material was unusual, it showed a superficial resemblance to that for triglycylglycine(G_4), but

but the patterns did not correspond on closer examination. The pattern also showed peaks at 31 A. and 14 A., i.e. low Bragg angles, see Sec. 17.. The specimen used for this trace had been obtained by centrifuging the material down onto a microscope cover slip, and so it may have been more oriented than usual. The hot water soluble Polyglycine II, diffractometer trace, obtained from a similar possibly oriented sample, showed a very strong 4.15 A. peak. The other Polyglycine II peaks were of reduced intensity compared with other traces. No peaks were observed at low Bragg angles. Since these crystallites, were shown to be thin hexagonal leaflets, this suggests that the 4.15 A. reflection is due to a set of spacings in the crystals which lies in the general direction at right angles to the plane of the hexagons. The mother liquor from this second hot water wash was treated with an equal volume of alcohol, a small deposit was obtained on standing. The diffractometer trace of this material, see Chart 10., showed Polyglycine II lines together with the two low angle spacings which had been previously observed. These substances which give rise to the low angle spacings may be low molecular weight

peptide phosphates, of large unit cell size. The hot water insoluble residue, remaining after the two extractions, was a mixture of Polyglycine I and II. When this residue had dried out in its centrifuge tube, the top portion was a much darker grey colour than the bottom. The diffractometer trace for this top crust, showed Polyglycine I lines and the weak 3.10 A. peak of Polyglycine II, but no sign of the very strong 4.15 A. peak.

b. It was thought that it might be possible to partially fractionate the products, by successive dilution of the phosphoric acid solution of the reaction products, with water. Thus 80 gm. of glycine and 16 ml. of phosphoric acid were heated together until the mass became dark brown, and the temperature rose to 170°C.. 150 ml. of hot water was added to the hot mass and the mixture brought to the boil, and filtered hot. The filtrate was allowed to stand and cool. The hot water insoluble residue was then extracted three more times with successive 150 ml. portions of hot water. All four of the filtrates deposited solids on cooling. This solid was centrifuged off, and the mother liquors allowed to stand overnight. Deposits were obtained from some

of these solutions. After centrifuging down these deposits, the mother liquors were then treated with an equal volume of ethanol. Deposits were obtained from all the solutions on standing. X-ray diffractometer traces were obtained to identify the products, see Chart 11.. The results obtained are summarised below.

Fraction 1.-

The hot water soluble material was Polyglycine II only. The deposit obtained on standing contained Polyglycine I. In addition strong lines were present corresponding to some of the strong lines in diketopiperazine, see Chart 9. . This trace may be compared with those obtained from the polymerisation of diketopiperazine in water, See Sec. 7.. Exactly the same lines appear strongly on both sets of traces, except for the line at 13° 48', which does not appear at all on this new chart. The extract from this solution on adding alcohol gave a material which showed peaks for Polyglycine I, and other peaks which it has not been found possible to identify at all. No other similar peaks have been found in any of the peptides or esters studied, see Sec. 15..

Fraction II.-

The hot water soluble portion showed lines for

both Polyglycine I and II . No deposit was obtained on standing. Treatment of the mother liquor with alcohol gave a deposit showing Polyglycine II lines only, see Chart 9..

Fraction III.-

The hot water soluble fraction showed Polyglycine I andII lines, but contained more Polyglycine II, than the Fraction II above. The deposit from solution on standing, and the deposit produced on adding alcohol are both Polyglycine II, the traces are identical, see Chart 11.

Fraction IV.-

The hot water soluble fraction was Polyglycine II only. No deposit was obtained on standing. The deposit obtained on adding ethanol was Polyglycine II only.

The hot water insoluble residue.-

This gave the diffractometer trace of a mixture of Polyglycine I and II.

c. Attempts were made to improve the yield of the reaction by heating under reduced pressure. This was not successfull.

d. The most certain method of obtaining Polyglycine II only from this reaction is to increase the

concentration of phosphoric acid with respect to the concentration of glycine. It was found that if 20 gm. of glycine and 10 ml. of acid were heated together, all the glycine dissolved in the acid, Heating was continued until the mass became dark brown. It was found that all the material obtained was soluble in hot water, and possessed the Polyglycine II type structure. The yield was 4 gm.(8%). The degree of polymerisation of this material is about 6. This would be expected from its solubility. Insufficient of the hot water soluble Polyglycine I was obtained to measure its degree of Polymerisation, but it must be close to that of the Polyglycine II, of low molecular weight.

e. POLYMERISATION OF GLYCINE AND PHOSPHORIC ACID IN SEALED TUBES.-

20 gm. of glycine and 5 ml. of 85% phosphoric acid was sealed up in a Carius tube, andheated in an air oven at 147°C., for 22 hours. The reaction mixture was extracted with hot water from the tube. The aqueous extract was boiled and filtered hot. The hot water insoluble residue was a mixture of Polyglycine I and Polyglycine II. The hot filtrate deposited a "leafing" solid on cooling. The

diffractometer trace showed that this also was a mixture of Polyglycine I and II. The mother liquor from this extraction deposited Polyglycine I only on standing. The yield of hot water insoluble material was 3.5 gm.(17%).

A second tube prepared as above, was heated at 170°C. for 21 hours. The product was extremely hard and difficult to remove from the tube. The hot water washed insoluble residue was almost pure Polyglycine I. The polymer was distinctly grey in colour. The hot water soluble fraction showed pronounced leafing, an X-ray trace, see Chart 11, showed this to be pure Polyglycine II. The electron microscope showed this material to consist of well defined hexagonal leaflets, see Sec. 14.. The crystalline quality of these leaflets was improved by recrystallisation from hot water. The total yield of polymer by this method is better than that obtained from glycine and hydrochloric acid. The yield of hot water soluble material is certainly greater. The purity of the Polyglycine I could be obtained by raising the temperature of the reaction . I t was found that when the hot water insoluble material, which had been hot water washed, was dissolved in saturated aqueous

calcium chloride solution, it was not completely soluble. A little of the material remained undissolved. This suggests that the material contained traces, of higher molecular weight polymers than the normal Polyglycine I and II. Any water soluble by products or lower molecular weight material would have been removed on extraction with hot water. This has only been observed with the material obtained from the experiments carried out in sealed tubes. The quantity of material obtained was too small to be separated and studied.

A STUDY OF THE TRANSITION BETWEEN POLYGLYCINE II AND POLYGLYCINE I.

SUMMARY OF RESULTS.-

The polyglycines were first heated dry in the open and sealed in glass tubes. There was no interconversion of form in either case. There was a 3% loss of weight in the case of both polymers, on heating in the open. This was at first thought to be due to the moisture regain of the polymer. On repeating the heating in a sealed tube, similar losses were noted, but the polymer did not appear at all damp, and there was no sign of moisture in the tube. The tubes did show some pressure on heating. The X-ray photographs showed that there had been no change of crystalline form and no appreciable decrease in crystalline quality. The polymers were discoloured after heating, as though some decomposition had occurred. Since the product was not homogeneous, as regards its chain length, it was assumed that some of the lower peptides which were known to be present had been decomposed.

The conversion of Polyglycine II into Polyglycine I occurred at all temperatures above 72°C. on heating

in a sealed tube with water. The rate of conversion at 72°C. was very slow, less than 50% complete after six weeks. Raising the temperature of the reaction from 72°C. to 120°C. produced a substantial increase in the rate of reaction. Raising the temperature by a further 30° to 150°C. produced approximately a six fold decrease in the time of reaction. Almost pure Polyglycine I was obtained after 27 hours heating.

The comparitively large temperature range over which the conversion occurred, supported the idea that Polyglycine II is a metastable form, and that there was no true transition between the two forms. Also the conversion occurred only in one direction, Polyglycine II being converted to Polyglycine I. It was not found possible to reverse the conversion between the limits of 20°C. and 160°C.. Neither was it found possible to completely remove the Polyglycine II X-ray lines, completely from Polyglycine I samples, by heating in water. It is suggested that the stable form of some of the lower peptides(D.P's 6-12), which are present in this product, is the Polyglycine II type structure. It has been shown that the lower water soluble peptides crystallised in this form. It is also shown in this section that if the

stock Polyglycine I, is heated in water at 100°C., and the solution filtered hot; on cooling a solid crystallised out. This solid showed the "leafing" effect, and had the structure of Polyglycine II, as shown by the X-ray photograph. The electron micrographs of this material, have shown that the crystals were thin hexagonal leaflets, or near hexagonal in form. The electron micrographs of these plates, or leaflets, are very similar to those obtained by Meggy and Sikorski(1956)[91], and show the same step growth. This agrees with their sug estion that although the polymer was derived from Polyglycine II, it was in fact of lower molecular weight, and chain length, than true Polyglycine II. This is certainly true of the material obtained here, since it is soluble in hot water.

Heating Polyglycine I in a saturated aqueous solution of calcium chloride, hydrolysed the polymer completely, no solid residue was obtained. Heating in a 20% or a 10% aqueous calcium chloride solution, did not alter the structure of the Polyglycine I. There was no increase in the intensity of the Polyglycine II lines on the X-ray photograph. The Heating of Polyglycine II in a saturated aqueous

calcium chloride solution, also produced no solid products. Heating in a 20% solution left a water insoluble residue. The X-ray photograph of this, showed, that a little of the Polyglycine II had been converted into Polyglycine I. Heating in a 10% solution resulted in a product, which showed the X-ray lines for Polyglycine II only. No conversion having occurred in this case. The concentrations of the calcium chloride solutions were carefully chosen. It was found during the precipitations of solutions of Polyglycine in saturated aqueous calcium chloride, that the Polyglycine II obtained, began to be precipitated, when the concentration of the calcium chloride solution was about 18%. Thus at about the 20% concentration, the polymer was soluble in the calcium chloride solution, whereas with the 10% solution the polymer was insoluble. Thus it seemed that the Polyglycine II must first dissolve before undergoing the change of form. The Polyglycine II is however hydrolysed in 20% calcium chloride, even at 72°C.

Heating the Polyglycines in 100% glycerol solution gave exactly the same results as heating in water. The Polyglycine II being extensively

converted into Polyglycine I. The loss of polymer was much less using glycerol, than in the corresponding reactions using water. The change was again irreversible, Polyglycine I remaining unchanged on heating in the glycerol. The X-rgy lines for the residue obtained by heating Polyglycine II in glycerol, showed lines for glycine, this also agrees with the results obtained using water.

Heating in anhydrous formic acid, resulted in some slight conversion of Polyglycine II, into Polyglycine I. Heating Polyglycine I in this medium resulted in no change of form. It was also noticed that the Polyglycine II was very readily attacked by the formic acid, being hydrolysed to glycine. The formic acid did not react as easily with the Polyglycine I. This again is in agreement with the idea that Polyglycine II is the metastable, and thus more reactive form of Polyglycine.

DISCUSSION OF RESULTS.-

It is significant that the conversion of the Polyglycine II to Polyglycine I is not reversible, This fact, and the large temperature range over which conversion can occur strongly suggests that the

Polyglycine II is the metastable form of the polymer, this would agree with the conclusions reached in Sec. 1.. There is certainly no true transition temperature between the two Polyglycines. It is also significant that no conversion occurred on heating Polyglycine II in the dry state, it seems that the conversion is dependent on the Polyglycine II being dissolved in solution, and being reformed as Polyglycine I, either in the solution, or when it comes out of solution. Although it is generally assumed that the two Polyglycines are identical when in solution. Thus the water or glycerol acts as a catalyst for the conversion of form.

The presence of the low molecular weight Polyglycine II, in the stock Polyglycine I may explain why it was found impossible to completely convert all the Polyglycine II to Polyglycine I. It may be that some of this low molecular weight polymer is stable at high temperatures in the Polyglycine II form. The loss in weight on heating the polymers in the dry state may be due to the decomposition of lower molecular weight polymers. The conversion on heating in calcium chloride solution also showed that the Polyglycine II dissolved before conversion.

EXPERIMENTAL.-

a. Heating the Polyglycines in the open air.-

Samples of Polyglycine I and II were heated in an air oven at 140°C. for periods of time up to 4 weeks. The X-ray powder photographs for both polymers showed no change in either the position of the lines or their intensities. The samples retained their own structures. The samples heated for a week or more, became a buff colour, this discolouring was more pronounced in the case of Polyglycine I. This suggested that some decomposition had occurred. Heating for 4 weeks, resulted in significant losses of weight of the polymer. These losses were 3% for Polyglycine I, and 5% for Polyglycine II. This work agreed with the work of Meggy and Sime(1956)[90], who found that the absorption of dye decreased on heating the polymer, but that the crystal structure remained unchanged.

b. Heating dry in sealed Carius tubes.-

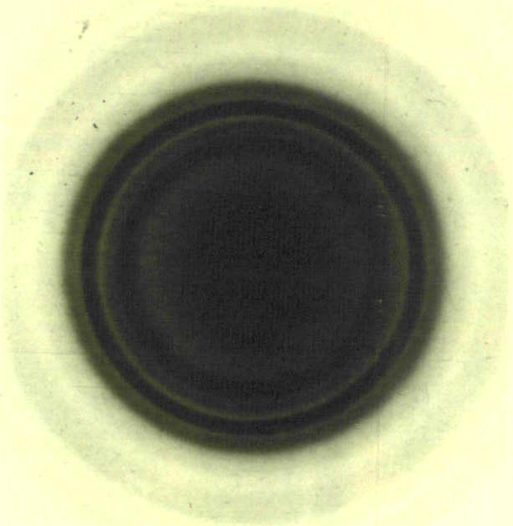
Both Polyglycines were heated dry in sealed tubes for three weeks at 140°C.. Again no change in the X-ray diagrams of either polymer was observed. The products assumed similar colours to those obtained on heating in the open. In both cases the loss of polymer was about the same as obtained on

heating in the open. There was some internal pressure in the tubes when opened in the cold. The contents of the tubes were quite dry, and there was no moisture condensed on the tube walls. The loss in weight could not therefore be accounted for as a loss of water. It is assumed that on prolonged heating some of the polymer decomposed, thus accounting for the reduction in the dye absorption. The polymer which decomposed was probably the lower molecular weight components of the non homogeneous Polyglycines.

c. Polyglycine I heated with water in a Carius tube.-

2 gm. of Polyglycine I, ex glycine and hydrochloric acid, was sealed up together with 1 ml. of water. The Carius tube was heated for 19 hours at 140°C. The X-ray diagram of the water and alcohol washed product showed no change in the Polyglycine I structure. There was however a slight reduction in the intensity of the lines due to Polyglycine II, which was present as an impurity.

1 gm. of Polyglycine I and 5 ml. of water was heated sealed for 3 weeks at 72°C. The X-ray photograph showed no change in the Polyglycine I structure.



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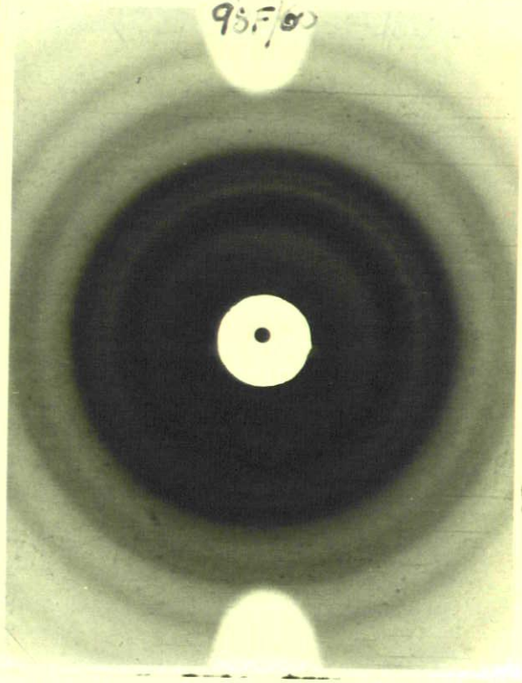

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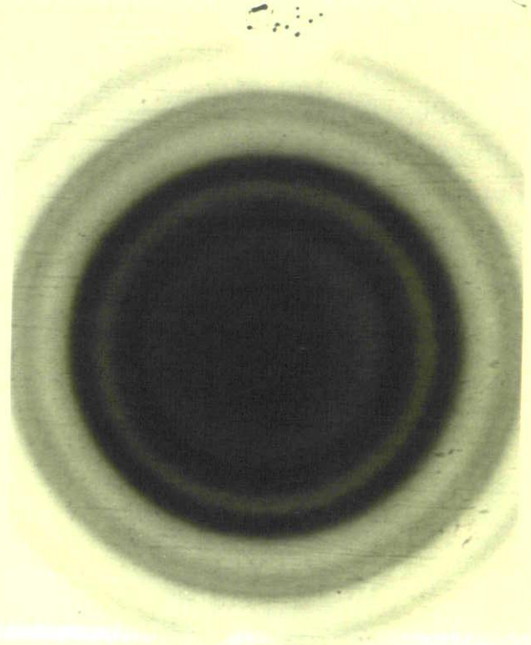

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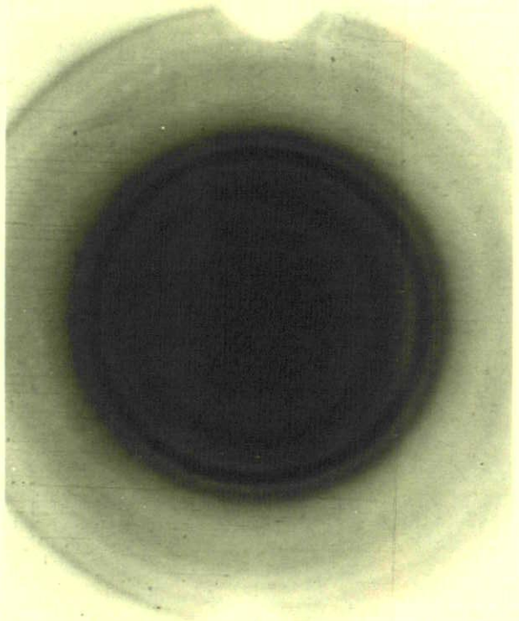

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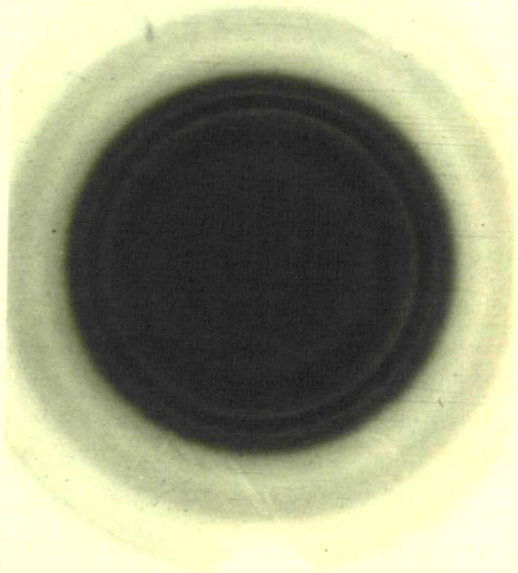


4

THE TRANSITION OF POLYGLYCINE II TO POLYGLYCINE I.

ONE GRAM PORTIONS OF POLYGLYCINE II WERE HEATED SEALED TOGETHER WITH 5 ml. PORTIONS OF WATER.

1. 2 WEEKS, AT 72°C.
2. 3 " , " 72°C.
4. 6 DAYS , " 120°C.
5. 27 HOURS, " 150°C.

d. Polyglycine II heated in water in a Carius tube.-

1 gm. of Polyglycine II was heated in a sealed tube together with 5 ml. of water at 72°C. for 2 weeks. The product was washed with water and alcohol, and dried over calcium chloride in vacuo. The X-ray diagram showed that no change in structure had occurred.

1 gm. of PolyglycineII and 5 ml. of water was heated sealed at 72°C. for 3 weeks. The product showed a weak X-ray line at 3,45 A. characteristic of Polyglycine I. Thus some of the polymer had changed its structure, butthe change was by no means complèèe.

1 gm. of Polyglycine II and 5 ml. of water was heated seàled at 120°C. for 6 days. The X-ray diagram of the product again showed that conversion to Polyglycine I had occurred, butthis conversion was incomplete, the 4.15 A. line for Polyglycine II was still very strong.

1 gm. of Polyglycine II and 5 ml. of water was heated sealed at 150°C. for 3 days. The X-ray showed that the intensity of the 4.4 A. and 3.45 A. lines for Polyglycine I was less than those in the prevàèus example. Thus raising the temperature by 10°C. had not appreciably altered the rate of reaction.

1 gm. of Polyglycine II and 5 ml. of water was heated sealed at 150°C. for 27 hours. The X-ray photograph of this product showed only a faint trace of 4.15 A. spacing of Polyglycine II, in the 4.4 A. band for Polyglycine I . Raising the temperature by a further 20°C. had made a considerable difference to the rate for the conversion of the PolyglycineII.

e. The hydrolysis products of the Polyglycines on heating in water.

The heating with water of the Polyglycines was repeated to determine the products of the hydrolysis of the peptides. Some 10% of the polymer was lost on heating in the previous reactions. There was no appreciable pressures in the cold tubes, and the solid was not discoloured, as in the case of dry heat. Thus it was assumed that hydrolysis had occurred.

2 gm. of Polyglycine I and 2 ml. of water was heated sealed at 140°C. for three weeks. The yield of water insoluble, unchanged, polymer was 0.97 gm.. The X-ray photograph of this material showed only Polyglycine I lines as expected. The solution decanted from this residue was allowed to stand.

Often fluffy precipitates had been deposited from this solution. The precipitate obtained in this case was centrifuged off and gave only enough material for an X-ray specimen. The spotted photograph obtained compared well with that for diketopiperazine. Thus at least some of the Polyglycine I is decomposed to diketopiperazine.

Polyglycine II was heated with water, 2 gm. and 2 ml., in a sealed tube at 140°C., for 3 weeks. The yield of insoluble polymer was 0.65 gm. again showing considerable loss. The centrifuged residue was used as an X-ray specimen, whilst still wet, before washing. The photograph showed that some of the polymer had been converted into Polyglycine I, and some glycine was also present in the product. There was no trace of any diketopiperazine. The solution decanted from the centrifuged product did not give a precipitate on standing. This seemed to indicate that the mechanism of hydrolysis is different in the case of Polyglycine II, and glycine and not diketopiperazine is the end product.

These results were incidental to the main investigation and have not been followed up. It is recommended that the results should be followed up as the effect has been repeatedly observed.

f. The effect of heating the Polyglycines in aqueous calcium chloride solution.-

1 gm. portions of Polyglycine I were heated with 5 ml. portions of saturated, 20%, and 10% aqueous solutions of calcium chloride at 72°C. for 7 weeks, 2 weeks, and 7 weeks respectively. The heating was carried out in sealed tubes. In the case of the saturated solution, the polymer dissolved completely, after about 2 weeks, there was no solid residue on cooling, and no precipitate on adding water to the reaction mixture. The polymer had been completely hydrolysed. The 20% solution did not dissolve all the polymer, this concentration was chosen as that at which precipitation of solutions of the Polyglycines, in calcium chloride solutions, commenced. The solid residue was centrifuged off, and washed with 20% calcium chloride solution, then water, and then ethanol, and dried over calcium chloride in vacuo. The yield was 0.56 gm.. The X-ray photograph showed that there had been no increase in intensity of the Polyglycine II, impurity, lines. Heating in the 10% solution also showed no change in the X-ray pattern.

1 gm. portions of Polyglycine II and 5 ml. portions of saturated, 20% , and 10%, solutions of aqueous calcium chloride, were heated sealed at 72°C., for 7 weeks, 2 weeks, and 7 weeks respectively. The polymer dissolved completely in the saturated solution, and no insoluble material could be obtained on precipitation of the solution with water. Again hydrolysis had occurred. The polymer was not very soluble in the 20% solution. The yield of insoluble residue was 0.63 gm., the X-ray photograph of this sample showed strong Polyglycine II lines. A very weak 3.45 A line for Polyglycine I was also present. Thus some conversion of form had occurred. The polymer was not soluble in the 10% solution, the residue obtained showed only X-ray lines for Polyglycine II. Thus although this reaction brings about the conversion of the Polyglycine II, this conversion is not extensive at 72°C.. The rate of hydrolysis of Polyglycine II is rapid at 72°C., so this is not a practical method for interconversion.

g. THE POLYGLYCINES HEATED IN GLYCEROL.-

1 gm. of Polyglycine I, and 5 ml. of 100% glycerol. was heated open for 7 days at 72°C. The insoluble product gave the X-ray lines for Polyglycine I only.

2 gm. of Polyglycine I and 2 ml. of 100% glycerol was heated sealed at 170°C. for 2 weeks. The X-ray photograph for the insoluble residue showed lines for Polyglycine I only.

1 gm. of Polyglycine II, and 5 ml. of 100% glycerol, was heated in the open at 72°C. for 7 days. The X-ray photograph of the insoluble residue showed lines for Polyglycine II only.

2 gm. of Polyglycine II and 2 ml. of 100% glycerol was heated in a sealed tube at 170°C. for 2 weeks. The water insoluble residue gave the X-ray pattern for a mixture of Polyglycine I and II, showing considerable conversion of form had occurred. The photograph also showed spotted lines for glycine.

The action of glycerol did not differ from that of water, except that the loss of polymer was much less. The yield in both cases was 1.9 gm.. The polymer was pure white, and the solution only slightly coloured.

h. TREATMENT OF THE POLYGLYCINES WITH ANHYDROUS FORMIC ACID.-

0.2 gm. of Polyglycine I was added to 4 ml. of anhydrous formic acid, in the cold. The mixture was allowed to stand. The insoluble polymer residue was cenrtifuged off. The X-ray photograph for this showed Polyglycine I lines only. The solution which had been decanted off, was treated with water and allowed to stand, and then alcohol. No solid was deposited in either case. There was little loss of polymer.

0.2 gm. of Polyglycine I was added to 4ml. of anhydrous formic acid, and the mixture heated to dissolve the polymer. The hot solution was allowed to stand overnight. A small quantity of deposit was centrifuged off. This gave the X-ray pattern for Polyglycine I only. The solution was treated with water and alcohol but did not yield a further deposit, the loss of polymer was about 75%.

0.2 gm. of Polyglycine II was added to 4 ml. of formic acid, in the cold, much heat was evolved, and about 75% of the polymer dissolved. The centrifuged insoluble residue was a mixture of Polyglycine II and a little Polyglycine I. The X-ray

photograph also showed spotted lines for glycine. The solution which had been decanted, did not deposit a residue on dilution with water. The loss of polymer was about 90%.

0.2 gm. of Polyglycine II and 4 ml.of formic acid were mixed and heated. The hot solution was allowed to stand overnight, a very small quantity of solid was deposited. The X-ray photograph of this solid showed strong lines for Polyglycine II, and a weak 3.45 A. line for Polyglycine I. Addition of water and alcohol to the decanted solution did not induce further precipitation. The loss of polymer was 80 to 90 % .

The conversion of Polyglycine II into Polyglycine I did not take place very readily in this medium. The formic acid hydrolysed the Polyglycine II very readily, but did not react so easily with the Polyglycine I. The product of the hydrolysis or decomposition was glycine.

1. STOCK POLYGLYCINE I HEATED IN WATER.-

The stock Polyglycine I obtained by the polymerisation of glycine in hydrochloric acid, was heated to boiling in water. The hot solution was filtered at the pump, through filter paper. The

solution did not filter easily or rapidly. The hot filtrate was allowed to stand and cool slowly at room temperature. After two or three hours a crystalline precipitate was obtained. This showed the "leafing" effect very strongly. This hot water soluble product was centrifuged down, washed with cold water and dried, in vacuo over calcium chloride. The X-ray photograph for the material showed lines for Polyglycine II only. Some of the uncentrifuged suspension in the mother liquor, was placed on an electron microscope grid. The crystallites were hexagonal plates, showing growth steps, see Sec.14.. This polymer was low molecular weight Polyglycine II, since it was hot water soluble. This material is thus present in all the stock Polyglycine I, prepared in this manner. The material is comparable with that obtained from glycine and phosphoric acid polymer preparations. This material is obtained in much lower yields than the glycine/phosphoric acid polymer.

THE ACID AND ALKALINE CHARACTER OF THE POLYGLYCINES.

SUMMARY OF RESULTS.-

Initial experiments with the aqueous suspensions of the two Polyglycines, and universal indicator, showed that the two polymers had distinctly different characters. The Polyglycine I suspension was acidic, pH. 4, whereas the Polyglycine II suspension was neutral, pH 7, to the indicator. The Polyglycine I, solid, was dyed pink by the indicator, the solid Polyglycine II, was not dyed at all. It was of interest to note that, Polyglycine I which had been heated, gave a brown colour, in the solution, when indicatorm was added to a suspension in water. The solution only became pink on heating, The colour given on heating, was a very dark pink, almost red. Polyglycine II which had been similarly heated, gave a green colour in aqueous suspension on adding universal indicator. The Polyglycine I had been affected by being heated. The Polyglycine II precipitated from aqueous zinc chloride, gave a green colotr, pH 7, in aqueous suspension with the indicator, on heating the suspension this colour became greenish yellow, pH 6.5 . The polymers had retained ions from the solutions in which they had

been prepared. A Donnan membrane effect was operative. Polyglycine I had retained hydrogen ions from the hydrochloric acid. The Polyglycine II, had retained hydrogen ions of either calcium or zinc, depending on the solvent used for its preparation. The Polyglycine II had been partially converted to the calcium or zinc salts. The calcium and zinc contents of the two Polyglycine II specimens were determined. The Polyglycine II precipitated from calcium chloride solution, contained one polymer molecule in every nine as the calcium salt. The polymer precipitated from solution in zinc chloride contained one polymer molecule in every three or four molecules as the zinc salt. This latter value was almost double the former value.

EXPERIMENTS.-

A suspension of 0.2 gm. of stock Polyglycine I in 5 ml. of distilled water, was treated with one drop of B.D.H. Universal indicator. A similar suspension of Polyglycine II was made up, and again one drop of indicator added. The heated Polymers had been heated at 100°C. for 13 weeks. Similar suspensions had been prepared and indicator added. In the case of Polyglycine II precipitated from

calcium chloride solution, the polymer had been partially converted to the calcium salt of the acid form of Polyglycine II. This had probably been caused by the production of some calcium oxide during the working up of the polymer. A similar explanation can also be applied to the partial zinc salt of Polyglycine II. It is also of interest that the Polyglycine II is not dyed by the indicator whereas the Polyglycine I is dyed. The release of hydrogen ions by Polyglycine I, and Polyglycine II, prepared from zinc chloride, was enhanced by heating.

THE DETERMINATION OF THE CALCIUM CONTENT OF POLYGLYCINE II.-

The Polyglycine II used was the stock polymer prepared from Polyglycine I. The Polyglycine I was dissolved in saturated calcium chloride solution and precipitated by an excess of water. It was washed three times with water and twice with alcohol, and dried over calcium chloride in vacuo.

Method.-

The average degree of polymerisation for this type of polymer is D.P.= 10. The calcium ions present would be attached to the polymer as the calcium salt of the carboxylic acid. The calcium

content expressed as a ratio of the polymer concentration would be.-

$$\frac{[Ca]}{[\text{polymer}]} = \frac{40}{588} = \frac{1}{147}$$

This would be the maximum value.

It was decided to use E.D.T.A. to determine the calcium content. Thus 1 ml. of 0.01 N. E.D.T.A. solution is equivalent to 0.4008 mgm. of calcium, or 10 mgm. of calcium ions require 25 ml. of this solution of E.D.T.A.. This is contained in 1.47 gm. of polymer, but since the calcium ion concentration was not in all probability a maximum, it was decided to use slightly more polymer, namely 1.75 gm.. This polymer was extracted with 10 ml. of 2N. hydrochloric acid, and centrifuged. 5 ml. of the supernatant liquid was pipetted off into a 50 ml. flask, and made up to the mark with distilled water. Ten millilitre aliquots of this solution were pipetted off. The 1ml. of 2N. acid which each of these contained was neutralised by the addition of 1 ml. of 2N. sodium hydroxide, a further 1 ml. of this solution was added to make the solution sufficiently alkaline. This solution was then titrated against the 0.01N. E.D.T.A. solution using murexide indicator. The titration was carried out using a micro-burette.

Samples were titrated directly with the E.D.T.A. solution, and also a back titration method was tried. The alkaline 10 ml. aliquot was treated with a pipetted 5 ml. portion of the E.D.T.A. solution, and the excess E.D.T.A. was back titrated with M/100. calcium chloride solution. A blank titration was carried out. Identical results were obtained by either method.

RESULTS.-

wt. of weighing bottle + P.G. II = 8.8517 gm.

wt. of ,, ,, + P.G. II finally =7.1050 gm.

wt. of Polyglycine II in the centrifuge tube =1.7467 gm.

This is the weight of polymer in 100 ml. of solution, in hydrochloric acid solution.

Titrations.-

Blank titration 0.07 ml. of E.D.T.A. solution required.

Direct titration of the polymer solution.-

10 ml. of the extract required 3.48 ml. of 0.01N. E.D.T.A. solution.

Back titration of the polymer solution.-

10 ml. of the extract + 5 ml. of the E.D.T.A. solution required 1.52 ml. of 0.01M. calcium chloride solution.

Thus the E.D.T.A. equivalent to the calcium in the extract was [5.0 - 1.52] = 3.48 ml.

Titration of the calcium chloride solution.-

10.0 ml. of the solution of 0.01 N. E.D.T.A. were required for 10 ml. of the calcium chloride solution. Thus the calcium chloride solution was truly 0.01M.

Calculation.-

The calcium in 10 ml. of the extract required [3.48 -0.07] = 3.41 ml. of 0.01N. E.D.T.A. solution This is equivalent to [3.41 x 0.4008] = 1.362 mgm. of calcium.

Thus 100 ml. of the extract would contain 13.62 mgm. of calcium, but this is contained in 1.7467 gm. of Polymer. Thus the ratiom of molecules of calcium to molecules of polymer is .-

$$\frac{[\ Ca\ ]}{[\text{polymer}\ ]} = \frac{13.62 \times 587}{4 \times 10^{4} \times 1.7467} = 0.1144$$

Therefore the[Ca]/[polymer] = 1 : 8.74

Thus only one polymer molecule in nine is present as the calcium salt, the remainder are present as the acid.

THE DETERMINATION OF THE ZINC CONTENT OF POLYGLYCINE II PRECIPITATED FROM SOLUTION IN AQUEOUS ZINC CHLORIDE.

The Polyglycine II used was the stock polymer, precipitated from a saturated solution of Polyglycine I in aqueous zinc chloride (70%) solution, by the addition of excess water. The polymer was washed with water and alcohol and dried over calcium chloride in vacuo.

METHOD.-

1.39 gm. of polymer was weighed out into a centrifuge tube. This was extracted with 10 ml. of 2N. hydrochloric acid, and centrifuged down. 5 ml. of the supernatant solution was pipetted off, and made up to 50 ml.. 10 ml. aliquots of this solution were withdrawn and diluted with 50 ml. of water. 5 ml. of ammonia and ammonium chloride buffer of pH 10 was added. The solution was titrated with 0.01N. E.D.T.A. solution, to solochrome black indicator. The end point being taken as a true clear blue colour of the solution. A micro-burette was used for the titration.

Results.-

wt. of weighing bottle + polymer initially = 8.3545 gm.

wt. of weighing bottle + polymer finally = 6.9562 gm.

wt. of polymer in the centrifuge tube = 1.3983 gm.

Titrations.-

Blank titration required one drop of 0.01 N. E.D.T.A. solution.

10 ml. of the extract solution required 6.8 ml. of the 0.01 N. E.D.T.A. solution.

Calculation.-

The zinc in the 10 ml. of the extract required 6.8 ml. of 0.01 N. E.D.T.A. solution.

Now 1 ml. of 0.01 N. E.D.T.A. is equivalent to 0.6538 mgm. of zinc.

Thus 100 ml. of the extract is equivalent to [6.8 x 10 x 0.6538] mgm. of zinc.

The number of moles of polymer per mole of zinc is.-

$$\frac{[\ Zn\ ]}{[\text{polymer}\ ]} = \frac{68 \times 0.6538 \times 588}{65.38 \times 10^{3} \times 1.3983} = 0.286$$

Thus the ratio is 1 : 3.504, or one to four . Thus one polymer molecule in three or four, is present as the zinc salt. This is double that shown for the Polyglycine II precipitated from calcium chloride.

THE SOLUBILITY OF THE POLYGLYCINES IN SATURATED AQUEOUS CALCIUM CHLORIDE.

The solubilities of Polyglycine I and II, have been determined in aqueous, neutral, saturated calcium chloride solution. Polyglycine I was not very soluble in the in the cold solution. Thus in order to adequately compare the solubilities of the polymers the determinations were carried out at 100°C. in a boiling water bath. 25 ml. portions of calcium chloride solution was pipetted into two boiling tubes, and placed in the boiling water bath. The polymer was weighed out by the difference method, and added to the solvent, with stirring. The addition of polymer was continued until a small quantity of the polymer remained undissolved. This method only gave an approximate value for the polymer solubilities, but in each case preliminary trial determinations, reduced the quantity of excess insoluble polymer to a minimum. It was impossible to centrifuge down the undissolved solid without cooling the liquid below 100°C.. The solution of either polymer was very slow at temperatures below 100°C, and so it would have been very difficult to determine the point at which solution ceased, at a lower temperature.

EXPERIMENTS.

a. Polyglycine I.-

The polymer used was the material obtained from the polymerisation of glycine in hydrochloric acid. The polymerisation mixture was heated at 140°C. for 24 hours in a sealed tube.

5.2765 gm. of Polyglycine I was added to the 25 ml. portion of calcium chloride solution, before the polymer ceased to dissolve. Thus the solubility of the Polyglycine I was 211 gm. per litre of saturated aqueous calcium chloride solution at 100°C.. This solution was irreversible, since Polyglycine II, was obtained on cooling the solution, and on precipitation of the solution by water.

b. Polyglycine II.-

The polymer was stock Polyglycine II, prepared by the precipitation of solutions of Polyglycine I in saturated aqueous calcium chloride solution at room temperature, by excess water.

12.1370 gm. of Polyglycine II was added to the 25 ml. portion of calcium chloride solution, before solution of the polymer ceased. The solubility of Polyglycine II was 485 gm. per litre of saturated aqueous calcium chloride at 100°C. The dissolution in this case was reversible, Polyglycine II was

obtained on cooling, or on precipitation by water.

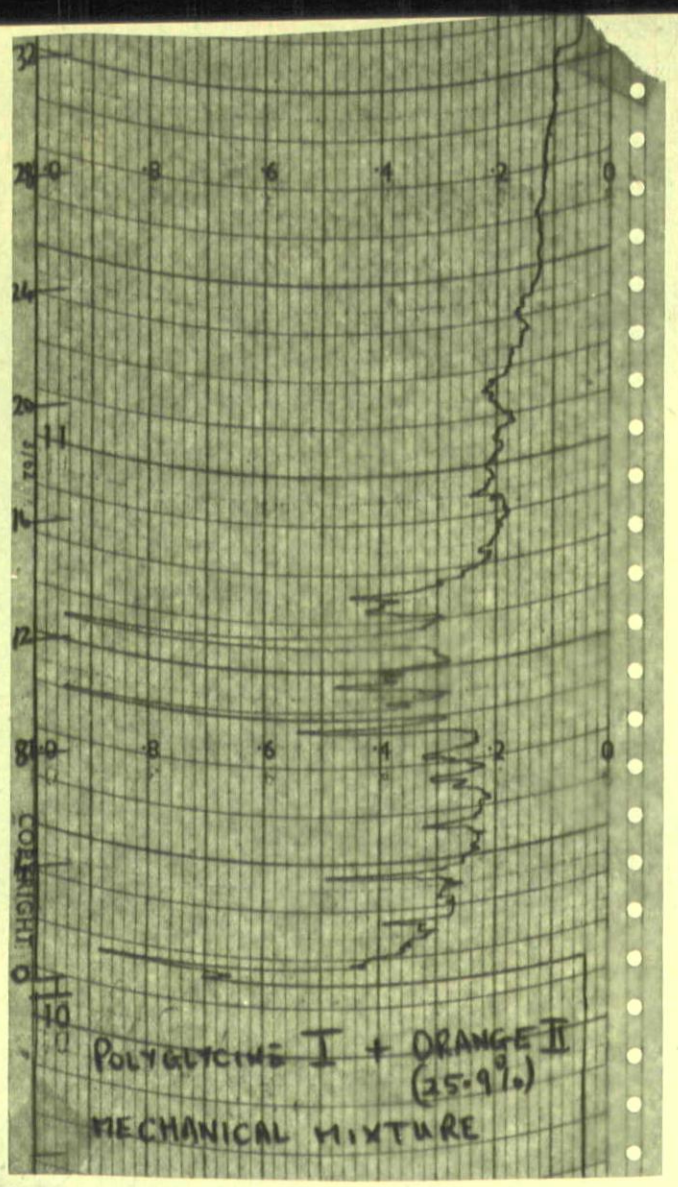
Thus the Polyglycine II was about twice as soluble as the Polyglycine I. This would be expected if Polyglycine I were the stable form of the polymer, and Polyglycine II the metastable form. This is in agreement with the work on the formation of the polymers, and on their interconversion in various solvents.

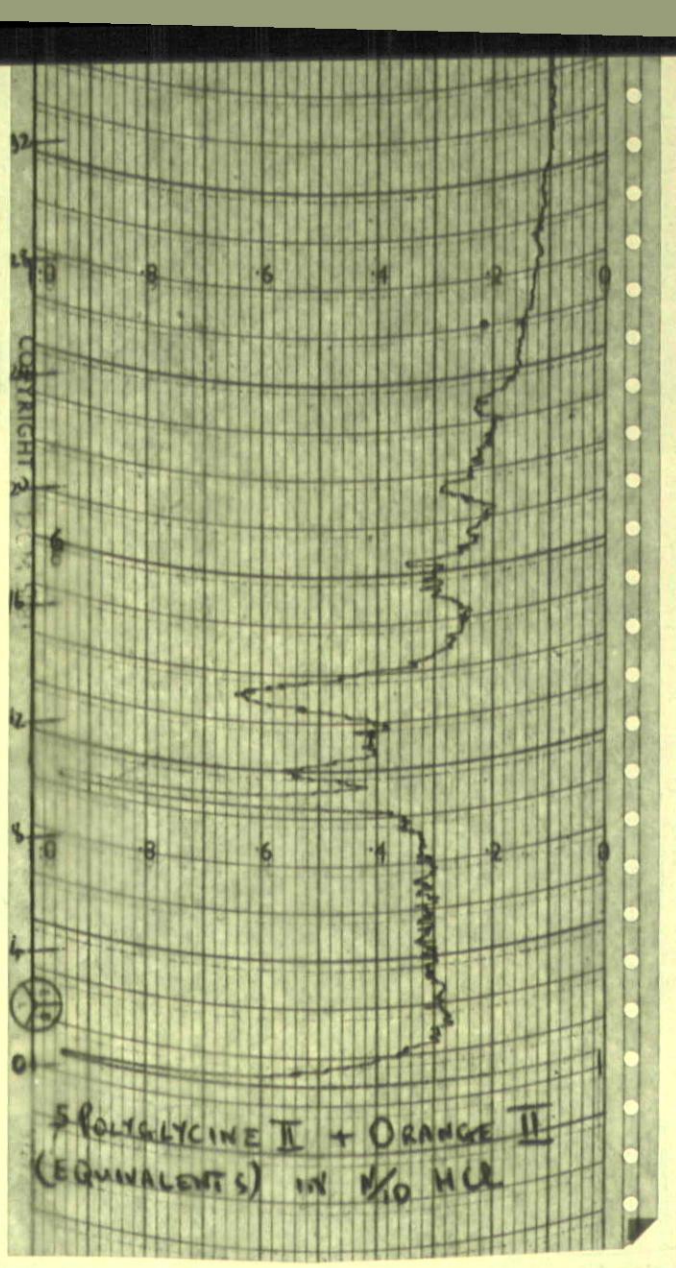
THE DYEING OF THE POLYGLYCINES WITH ORANGE II.

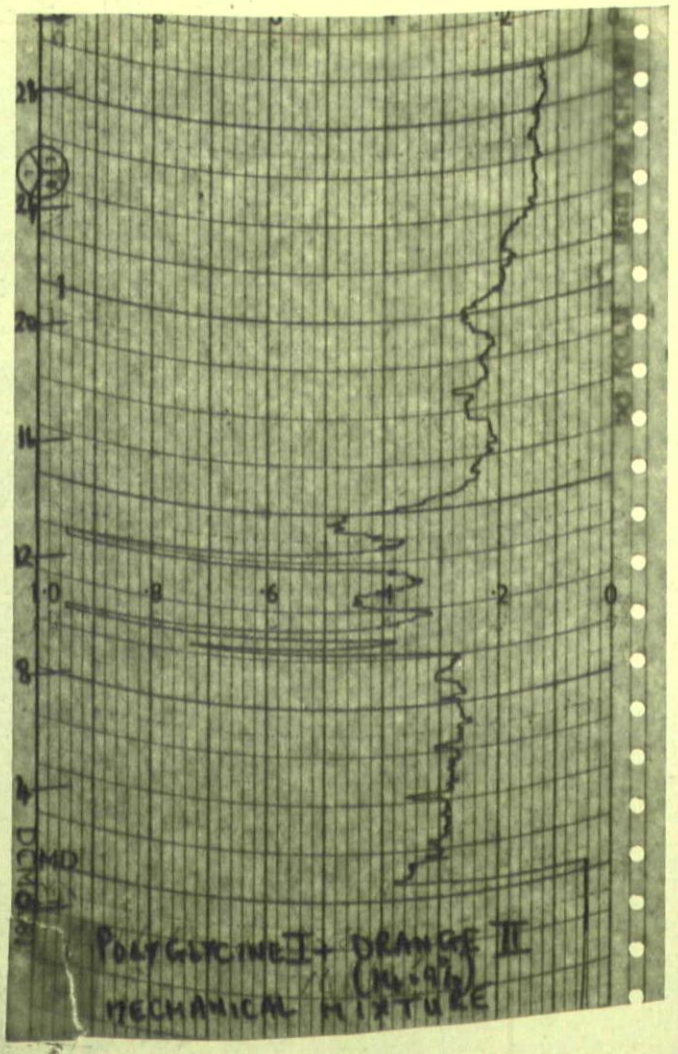
This work has been carried out as a follow up to the work of Meggy and Sims(1956)[90]. These workers showed that Polyglycine I absorbed only 42% of the theoretical quantity of orange II dye, whereas Polyglycine II absorbs orange II equivalent to the total terminal amino groups in the polymer. They also showed that the X-ray diagrams of the dyed polymers were identical with those of the undyed polymers.

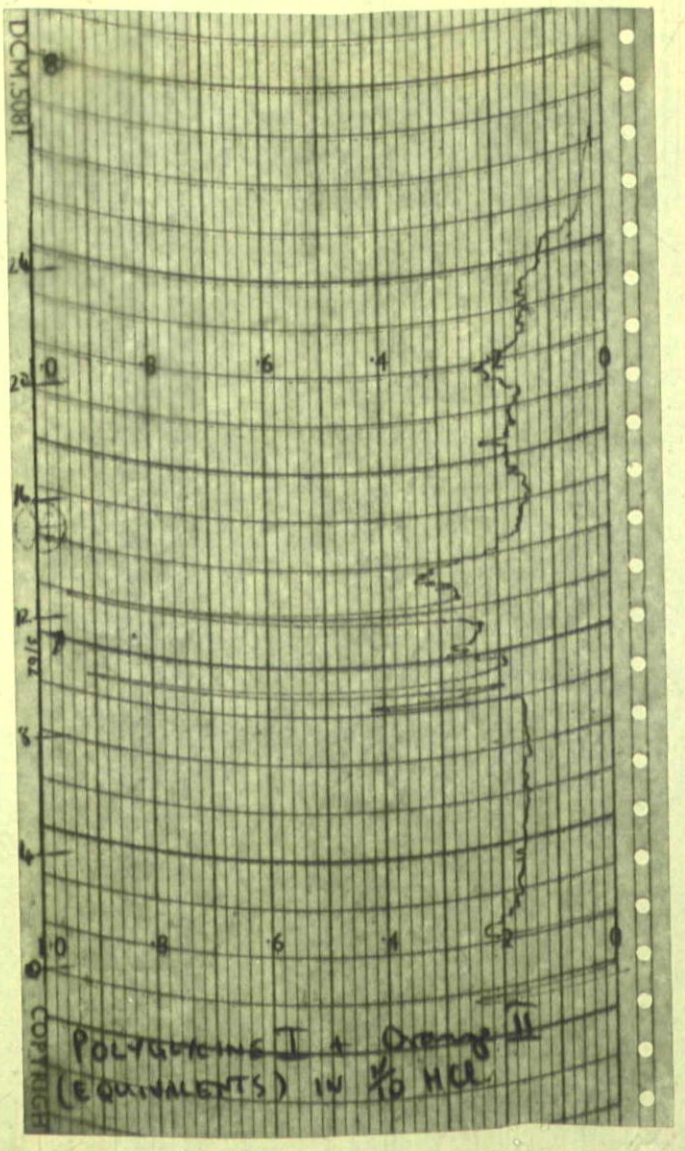
The polymers used in the dyeing experiments were washed with a sodium acetate and acetic acid buffer of pH 5.1 . The polymers used were stock Polyglycine I, ex glycine and hydrochloric acid; Polyglycine II, ex calcium chloride, and Polyglycine II ex zinc chloride.

0.05, 0.01, and 0.2 gm. portios of each polymer were treated with 10 ml. of a solution of the sodium salt of orange II, containing 3.5 gm. per litre. The mixing of polymer and dye solution was carried out in a water bath at 80°C, from stocks kept in the waterbath. The system was allowed to stand in the bath for 30 minutes after mixing. The suspension of dyed polymer was then centrifuged down, and washed once with water and once with ethanol.


POLYGLYCINE I + ORANGE II
(25.9%)
MECHANICAL MIXTURE

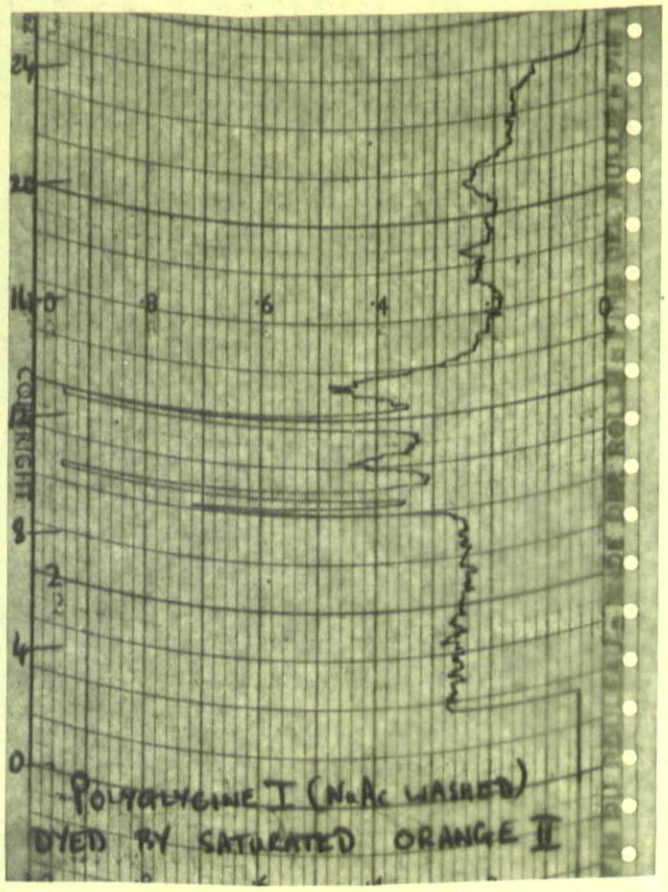

5 POLYGLYCINE II + ORANGE II
(EQUIVALENTS) IN N/10 HCl

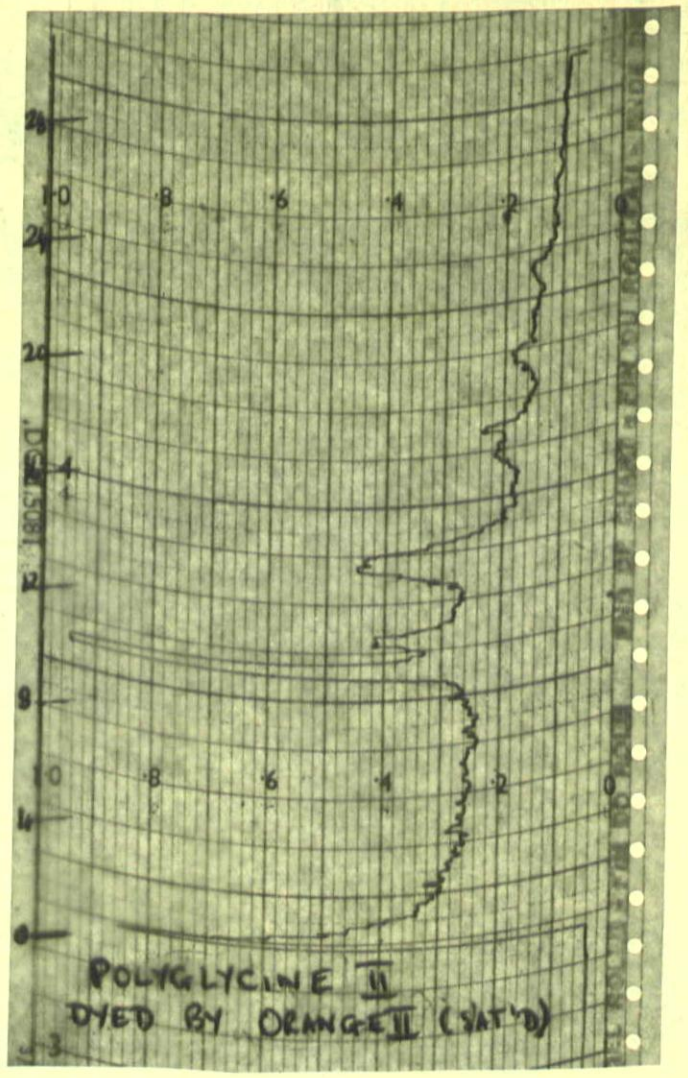

POLYGLYCINE II + ORANGE II
(26.9%)
MECHANICAL MIXTURE

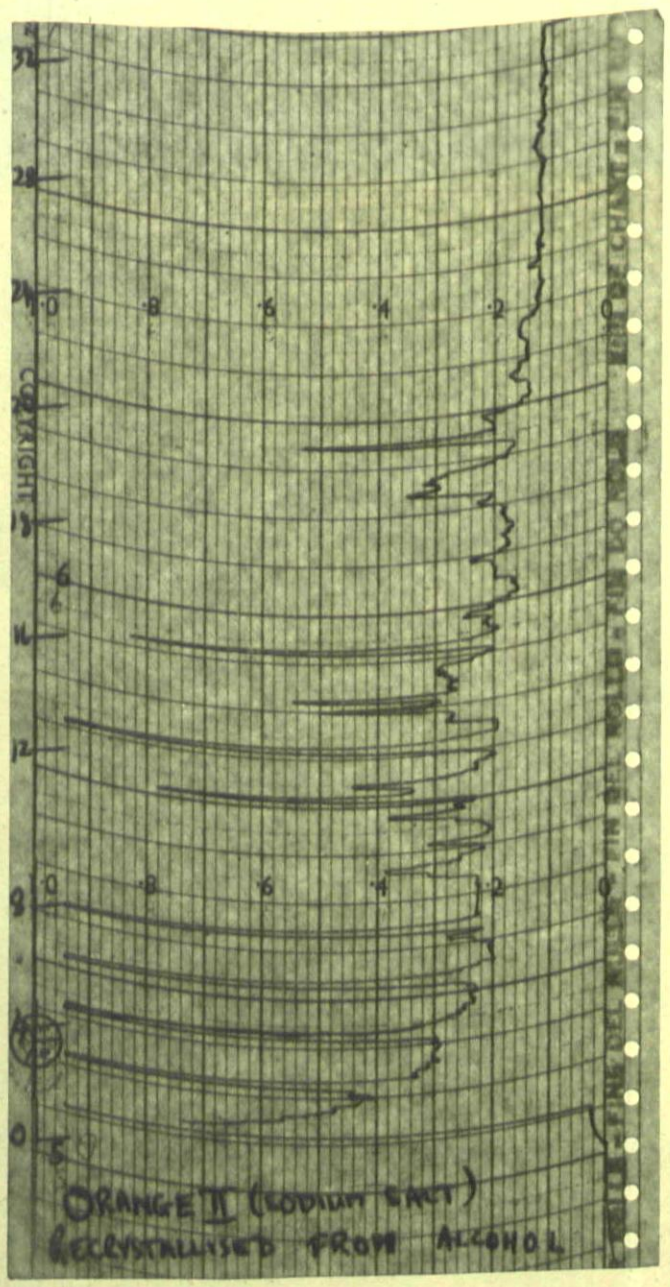

POLYGLYCINE II + ORANGE II
(EQUIVALENTS) IN N/10 HCl

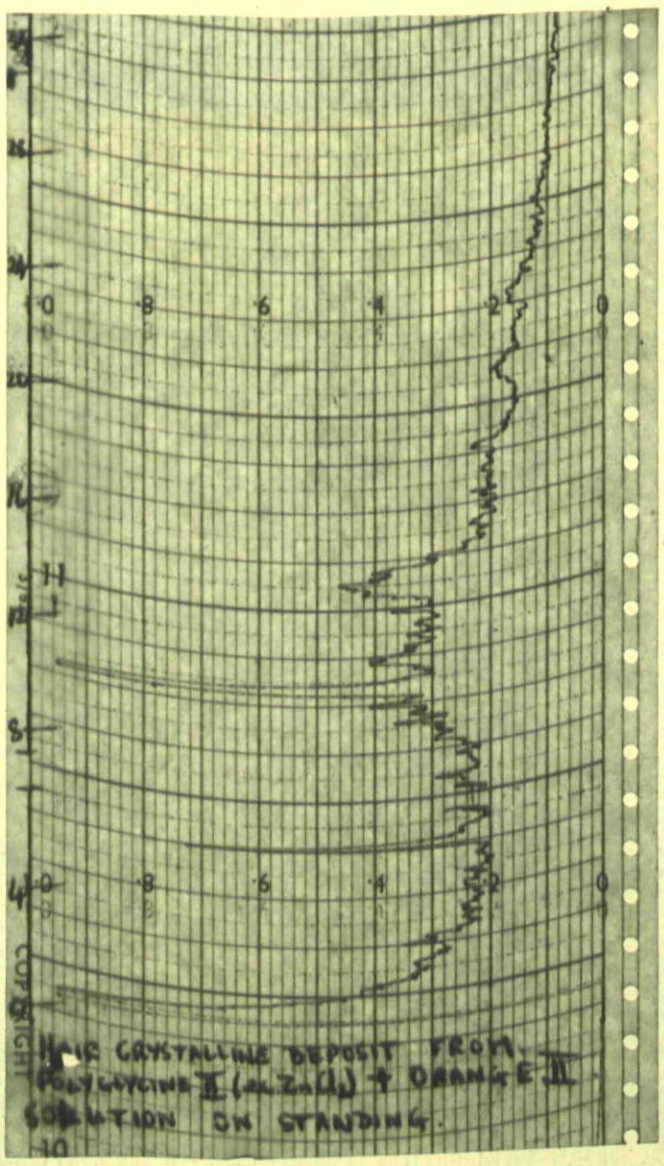
The dyed residue was dried in vacuo over calcium chloride.

The X-ray diagrams of all the samples showed no change in the X-ray diffraction pattern on dyeing. There was no alteration in the X-ray spacings of the polymers, and no trace of any lines due to orange II. This must mean that the dye neither disturbs the structure of the polymer, nor does it take up a regular arrangement in the polymer. Mechanical mixtures of dye and Polyglycine I were made up to see what concentration of randomly dispersed dye was necessary for its X-ray diffraction pattern to appear. It was found that Polyglycine containing 15% of dye was required to show an orange II diffraction pattern, the lines even then were very weak, and certain of the stronger orange II lines coincided with the Polyglycine I lines. The lines for a 30% mechanical mixture were by no means strong and could easily be lost by underexposure of a photograph. The X-ray diffractometer was used as a more sensitive means of determining the presence of orange II diffraction lines. The traces obtained with the above samples are shown opposite. The traces showed that the lower angle lines for orange II, did


POLYGLYCINE I (N.Ac WASHED)
DYED BY SATURATED ORANGE II


POLYGLYCINE II
DYED BY ORANGE II (SAT'D)


ORANGE II (SODIUM SALT)
RECRYSTALLISED FROM ALCOHOL


HAIR CRYSTALLINE DEPOSIT FROM
POLYGLYCINE II (sol. Zn Cl2) + ORANGE II
SOLUTION ON STANDING.

just appear on the 15% mixture of dye in polymer. The dyed polymers still did not show any alteration in position or intensity of the polymer peaks, and no peaks for orange II. These samples should have contained more than 25% of dye, and if this had been arranged regularly throughout the polymer lattice, diffraction peaks for its structure would have appeared.

The Polyglycine II, obtained from a solution in zinc chloride by precipitation with water, showed a peculiar phenomenon on dyeing. The solution containing the dyed polymer was allowed to stand. A deposit of orange hair like crystals was obtained. It was found that this deposit was soluble in much diluted supernatant solution, dilution was carried out with water, on warming but were reformed on cooling. An X-ray diffractometer trace of the hair crystals showed X-ray lines for Polyglycine II, together with many other weak lines, some of these lines corresponded to orange II lines. There is one strong line which corresponded to a strong line in the orange II trace,but the other strong lines of the orange II pattern did not appear as strong lines. This material is most probably a complex compound of the dye and low molecular weight Polyglycine II.

PAPER CHROMATOGRAPHY OF THE PRODUCTS OF THE POLYMERISATION OF GLYCINE IN HYDROCHLORIC ACID.

The products from the polymerisation of glycine in hydrochloric acid, ratio 5 gm. of glycine to 1 ml. of concentrated acid, were analysed by paper chromatography. The moving phase used was, butanol, acetic acid, and water, proportions 60:15:25. It was shown that the crude reaction product dissolved in a little water, gave a spot for unchanged glycine , with a long trail of spots for low molecular weight polymers, when sprayed with ninhydrin reagent. The paper was then chlorinated by suspension in gaseous chlorine for 10 minutes, allowed to stand in air overnight, and then dipped into a mixture of 1% starch and 1% potassium iodide solution, according to the method of Rydon and Smith(1952)[101]. It was found that the reaction product contained diketopiperazine, which appeared on the paper as a blue-purple spot when treated as above. The chromatogram was carried out with a diketopiperazine control spot. The accepted reaction mechanism for this polymerisation, ref. Meggy(1953,1956)[88,89]; is that polymerisation occurs via glycylglycine, the chromatograms showed that this was not necessarily the case. The original

glycine was chromatographically pure, so that the diketopiperazine must have been formed during the reaction. It was not possible to determine whether the reaction mixture also contained glycylglycine, since in this particular solvent system, the R_f value of glycylglycine was too close to that for glycine.

Butanol, pyridine, and water; proportions 1:1:1(upper phase), this did not improve the separation of glycylglycine and glycine enough.

Phenol, and water, proportions 1:1, was also tried as a solvent system. It was found difficult to use the chlorination method of spot detection, since the background colour was always too high. Spraying with ninhydrin showed that the trail which, with the first solvent, had followed the glycine spot; was now ahead of this spot. This would be expected if the trail were due to low molecular weight polymers, since the R_f values of the polymers would be much higher in this solvent system. Again the separation of the glycine and glycylglycine spots was not sufficient. Attempts were made to replace the chlorination method of detection, by another method. The papers were exposed to iodine vapour, this was not successful, again the background intensity

was too high. A method of detection which was found to be useable, was to heat the paper at 120°C. overnight. The polymers charred. But after this the spots would not react to any of the sprays, although the background intensity had been reduced.

The product from the polymerisation of glycine in phenol/water(lower phase) was similarly shown to contain diketopiperazine, and other unidentified spots of higher R_f values. The solvent used was butanol, pyridine, and water.

The product from the polymerisation of 80 gm. of glycine in 16 ml. of phosphoric acid, by heating in the open, was also shown to contain diketopiperazine. Using the same solvent as above.

The polymerisation of glycine in hydrochloric acid in sealed tubes was examined by making a chromatogram of the product obtained after various times of heating. After three hours only a very weak spot for diketopiperazine was detected. The chlorinated intensity of the spot increased slightly for heating times up to 6 hours. There was then a marked increase in intensity after 8 hours heating. this is the time in the reaction at which solid Polyglycine II is obtained. The solvent system used was butanol, pyridine and water. Diketopiperazine

was still present after 50 hours heating of the reaction mixture. The polymer had been almost completely converted to Polyglycine I by this time, see Exp. Sec. 9.. There was no reduction in the intensity of the spot after the sudden increase after 8 hours heating.

This work proves that diketopiperazine is produced during many of the direct polymerisation reactions of glycine. The work also shows that the diketopiperazine is produced during the preparation of Polyglycine II in the polymerisation reaction of glycine in hydrochloric acid. The diketopiperazine is not produced during the conversion of Polyglycine II into Polyglycine I, since it would not then have appeared so early in the reaction.

THE ELECTRON MICROSCOPY OF THE POLYGLYCINES.

SUMMARY OF THE RESULTS.-

One of the major aims of this work was to prepare hexagonal crystals of Polyglycine II, and to define the conditions necessary for their preparation. This aim has been achieved on the microcrystalline scale.

Polyglycine I prepared by any of the methods given in this thesis, appeared under the electron microscope as very small irregularly shaped particles. No definite crystal habits could be observed. The only Polyglycine I specimen which showed any distinct crystal shape, was that prepared by the slow polymerisation of glycine in phenol, Sec. 4. p. 5. These crystals showed rectangular cleavage, suggesting that at least two of the crystal axes were at right angles. The flat laminae were heavily striated, along their length. None of these thin laminae could be made to give electron diffraction patterns. The Polyglycine I prepared by other methods, always contained some very thin laminae showing Moire fringes, this material was only present in small quantities. These laminae could be made to produce hexagonal spot diffraction patterns. These laminae appeared to be Polyglycine II crystals present as an impurity, i.e. Polyglycine II which had not been converted into

Polyglycine I. Comparison of the material, and the diffraction patterns produced by it, with those for Polyglycine II showed that this was probable. Also on heating the Polyglycine I in water, to boiling, and filtering hot; a "leafing" crystalline deposit was obtained, on cooling the filtrate. This material was found to be a low molecular weight peptide, which had crystallised in the Polyglycine II structure, see Sec. 9, p.16 . The electron microscope showed that these leaflets were very thin hexagons. They appeared to be truly hexagonal, and also showed distinct growth steps. This material must have been present in the crude Polyglycine I, it could not have been produced during the brief boiling in water.

Fractional crystallisation of Polyglycine II, precipitated from solutions in calcium chloride solutions, ~~Seem~~ Sec.1, produced the best quality specimen of high molecular weight Polyglycine II. The crystals obtained were not weal defined hexagons, but they did show hexagonal growth steps. A similar fractionation was carried out from solutions in 70% aqueous zinc chloride solution. The products passed through a series of crystal shapes during the fractionation. The crude product consisted of "puff-balls", spherical particles, which were

spherical particles consisting probably of aggegates of crystallites, produced by the rapid precipitation. Reprecipitation of the material, produced "donuts", the centres of the"puff-balls" became increasingly transparant to the electron beam, whilst the edges of the particles remained thick. Some of the crystals appeared to have holes right through the centre, hence the comparison with the American ring doughnut. In the next stages of fractionation the "donuts" took up an hexagonal shape, with rounded corners, but the crystals still remained very thick. In the final stages the edges of the"donuts" appeared to grow outwards, this new growth was of the terraced, or stepped type. This growth appeared to occur at the expense of the thicker edges of the "donuts". The thick rough hexagons appeared to be the limiting form for this material, since further crystallisation produced no change.

The best crystals of hot water soluble,low molecular weight Polyglycine II, were obtained from the product of the polymerisation of glycine in phosphoric acid, see Sec. 8.. This material showed the Polyglycine II X-ray structure. The microcrystals obtained on recrystallisation of the crude material from hot water, were very thin

hexagonal leaflets, and showed very distinct growth steps. These crystals were extremeley well defined, and were of far better quality than could be obtained by the repeated precipitation of true Polyglycine II, from calcium chloride solution. This water soluble polymer would not give an electron diffraction spot pattern , with the plates lying in the plane of the film, or electron microscope grid.

The hot water soluble Polyglycine I, obtained from the glycine and phosphoric acid polymerisation, did not show any regular crystal shape under the electron microscope. This material was not however recrystallised since only a very small sample was obtained.

DISCUSSION OF RESULTS.-

The electrom microscope has shown that the hot water soluble material is the best crystalline sample which has been obtained for Polyglycine II. This indicates that this is the best method for obtaining macrocrystals of Polyglycine II, suitable for X-ray examination. The product from glycine and phosphoric acid polymerisation produced a greater proportion of this material than any other method. This material may also produce a well defined crystalline sample of water soluble Polyglycine I.

The electron diffraction results were extremely disappointing. The electron microscope used did not make it possible to accurately orient the specimen. The specimen could only be placed on the grid with its axis at right angles to the electron beam. The samples of Polyglycine II which did show electron diffraction spot patterns were too irregular to determine their orientation. It was significant that the water soluble Polyglycine II did not give a diffraction pattern when placed at right angles to the electron beam. This could mean that the axis in the direction of the electron beam, is not at right angles to the other two axes. Thus the Polyglycine II may not have an hexagonal structure but is possibly triclinic. A more thorough investigation of the electron diffraction patterns would be required at different orientations, before any definite conclusions could be reached.

EXPERIMENTS.-

a. Electron microscopy techniques.

The electron microscope used was the Phillips model E.M. 100B.. The kilovoltage used to study the Polyglycine specimens was 80 kv., unless otherwise stated. The specimens of both Polyglycine I and Polyglycine II, were found to be perfectly stable

in the electron beam at this kilovoltage.

b. Specimen preparation.-

The specimens were prepared using a replica technique. Drops of aqueous suspensions of the polymers were spread on pieces of freshly cleaved mica. The water was then evaporated off in a warm air oven. The dried mica squares were then placed in a shadowing unit. Thin films of carbon, and metal either gold or ~~platinum~~ palladium, were then deposited on the specimens under vacuum. The films were then floated off the mica squares on water, and collected on copper grids. This method was preferred since it gave more even particle distribution, than the method of dropping an aqueous suspension on to prepared carbon films supported on copper grids. This method is normally used to prepare replicas of the specimens. The actual specimen remaining behind, stuck to the mica, on floating off. The emthod was used in this case initially, with the intention of producing replicas, but it was found that the polymer adhered more readily to the carbon film, than to the mica. The method thus produced a normal specimen containing some replicas. The specimens were thus ideal for study,

NOTE.-

THE MAGNIFICATION OF THE ELECTRON MICROSCOPE PHOTOGRAPHS.

The photographs shown have a magnification of approximately 12,000x , except for the following photographs.

p.11a. top photograph, magnification 40,000x.

p.12a. left hand side photographs, magnification approximately 20,000x.

p14a. magnification 30,000x.

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by electron, diffraction, microscopy, or replicate techniques. The difference in optical density between even the thin specimens, and the surrounding metal and carbon film, was ample for one to see whether or not a replica was being examined.

c. Electron diffraction.-

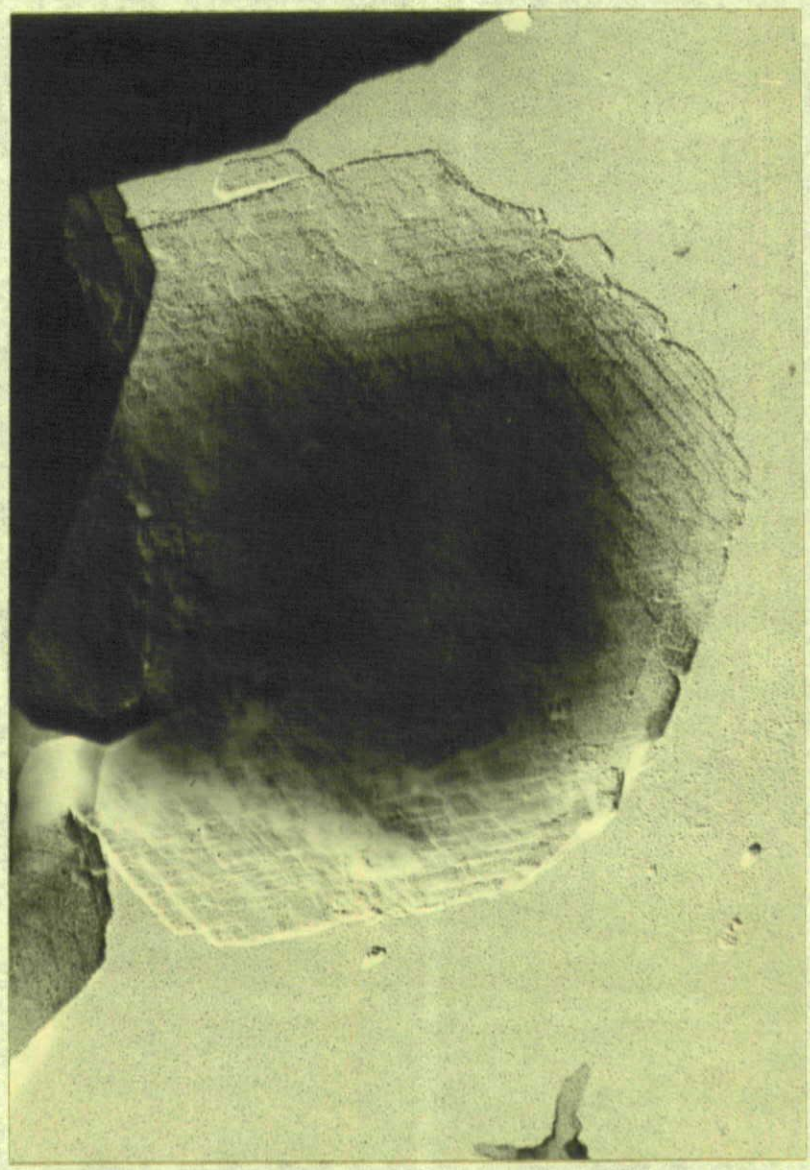
The electron microscope was equiped with special diffraction pole pieces, these gave much better results than the high resolution pole pieces. The specimen could only be rotated about an axis trough the diameter of the specimen grid. Thus the orientation was limited to one plane of the specimen, since the specimens were thin plates, and lay in the plane of the specimen grid. It was not possible to completely orient the single crystal specimens. This meant that a detailed study of the electron diffraction of the single crystals was not possible.

ELECTRON MICROSCOPY OF THE POLYGLYCINES.-

Meggy and Sikorski(1956)[91] obtained a single chance preparation of Polyglycine II, which when examined under the electron microscope showed thin hexagonal leaflet microcrystals. The exact details of this preparation were not known, but the material was thought to have been precipitated

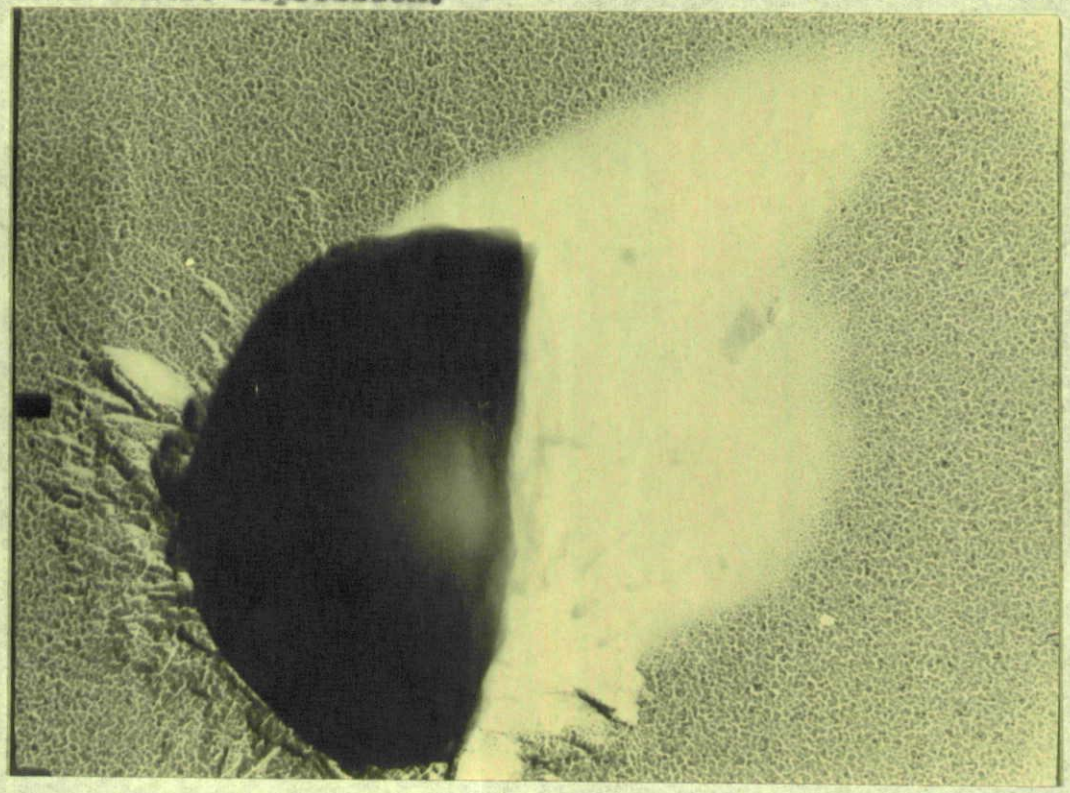
POLYGLYCINE II.-

Obtained by repeated reprecipitation by water, of solutions of the polymer in aqueous saturated calcium chloride solution. Crystals obtained from the seventh reprecipitation.



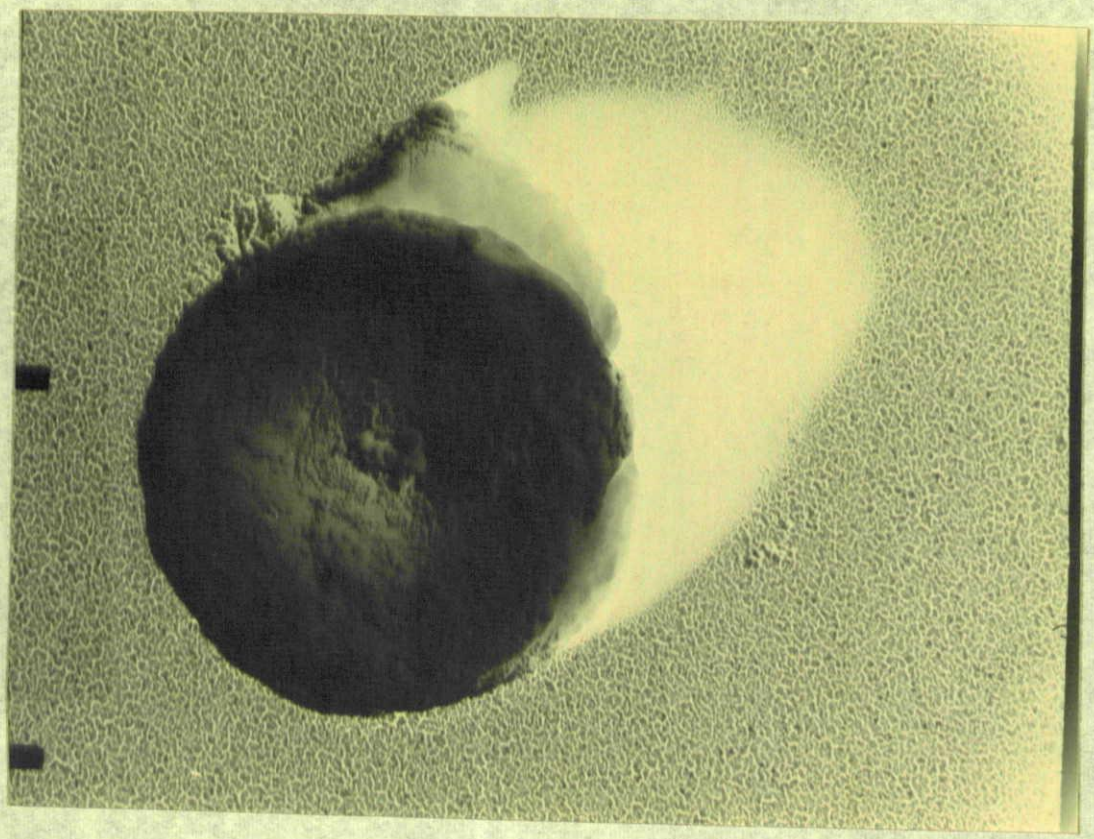
from a solution in saturated aqueous calcium chloride solution. Thus slow precipitation from calcium chloride solution, and zinc chloride solution was attempted, using water as the precipitant. It was found that if the precipitation was carried out slowly at 80°- 90°C., the initial precipitate showed considerable "leafing" effect, but on cooling the hot solution, or on centrifuging down the precipitate, the rate of nucleation was so great that these leaflets did not grow. The best method of preparing good crystals of Polyglycine II was by the repeated precipitation of the polymer from solutions in either calcium chloride or zinc chloride solution, by water. About six reprecipitations were required to obtain the material shown in the photograph opposite. These crystals were not very well defined hexagons in outline, but they did show hexagonal shaped growth steps, or terraces. This material was obtained using calcium chloride solution as the solvent. A similar set of precipitations was carried out using aqueous 70% zinc chloride as the solvent. The product showed a hexagonal outline, but the crystals were much thicker than had been obtained by the previous method.

HALF "DONUT" ex. Zinc chloride solution. shadow shows the centre depression.

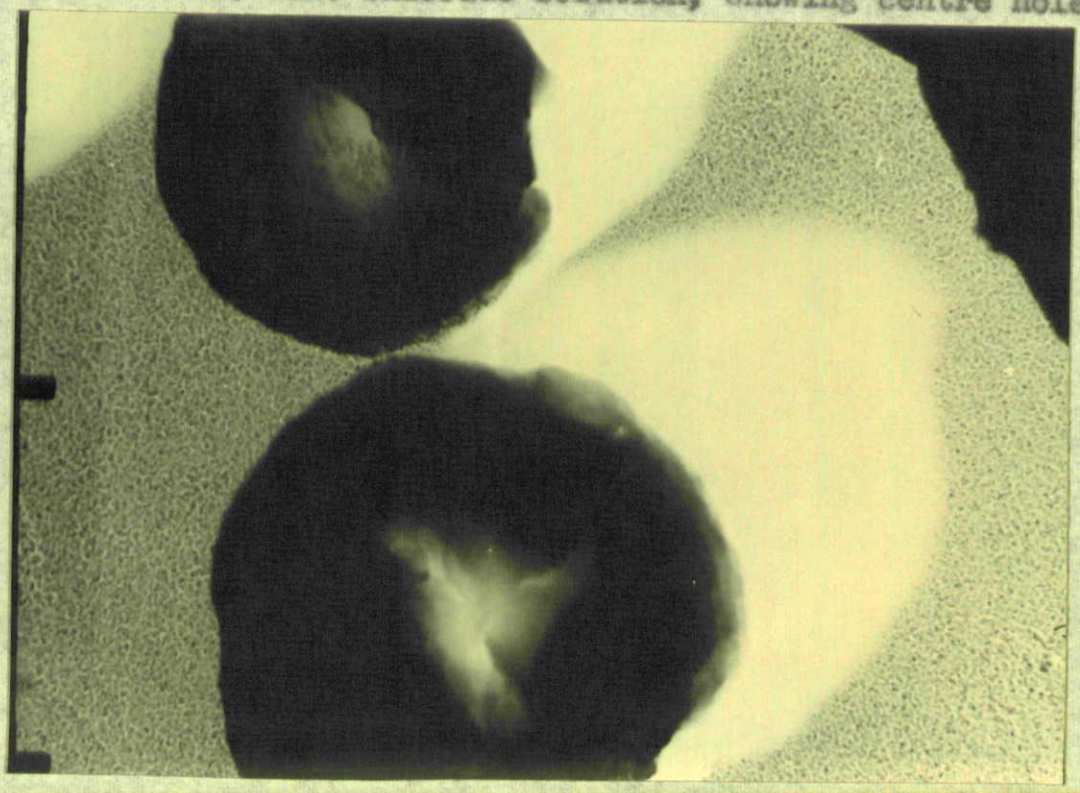


"DONUT" in process of conversion to HEXAGONAL PLATE ex. Zinc chloride solution.

"PUFF-BALLS" ex. Zinc chloride solution.

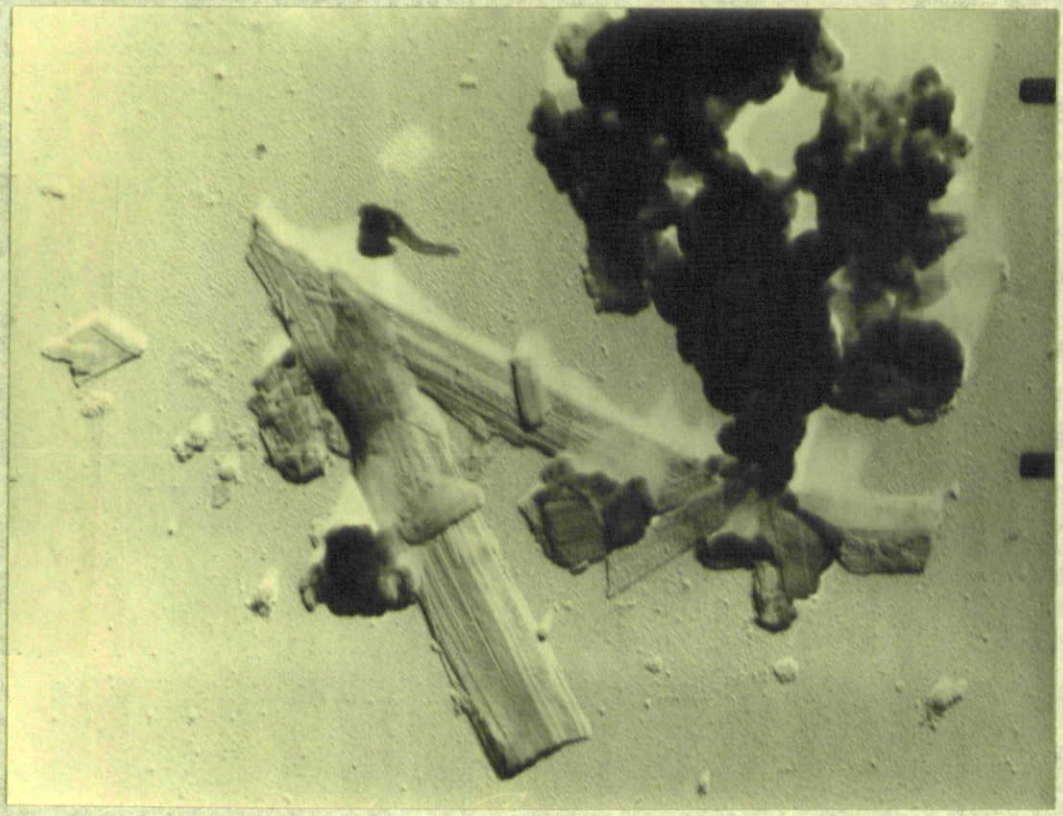


"DONUTS" ex. Zinc chloride solution, showing centre holes.



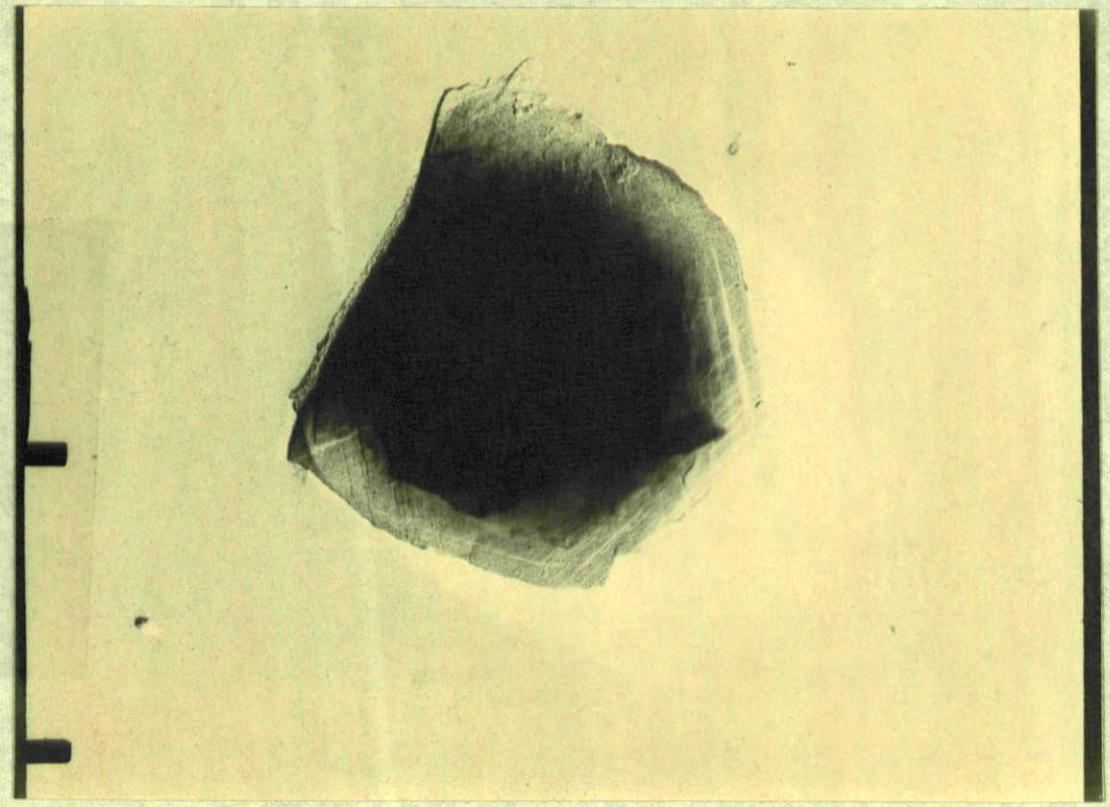
An examination was carried out of the forms in which the polymer was precipitated. It was found that the use of aqueous zinc chloride, instead of calcium chloride was found to make no difference to the crystal shapes of the products obtained on precipitation by water. It was found that the shapes of the microcrystals obtained differed according to the rate of precipitation, and the number of times which the precipitate had been recrystallised. A primary rapid precipitation of the polymer from the salt solution produced spherical "puff-balls", further reprecipitation whether fast or slow, converted these into "donuts". The centres of the "puff-balls" became increasingly transparent to the electron beam, whilst the edges remained thick. This was thought at first to be an optical illusion, but several "donuts" were found which had been split in half, the shadows showed distinct depressions in the centres. Some "donuts" appeared to have holes right through them. In the next stage, the "donuts" started to take up an hexagonal shape, definite sides and rounded corners appeared. Finally the edges of the hexagonal "donuts" appeared to grow outwards, this new growth being of the terraced type, and showing distinct hexagonal shaped terraces. This outward growth appeared to occur at

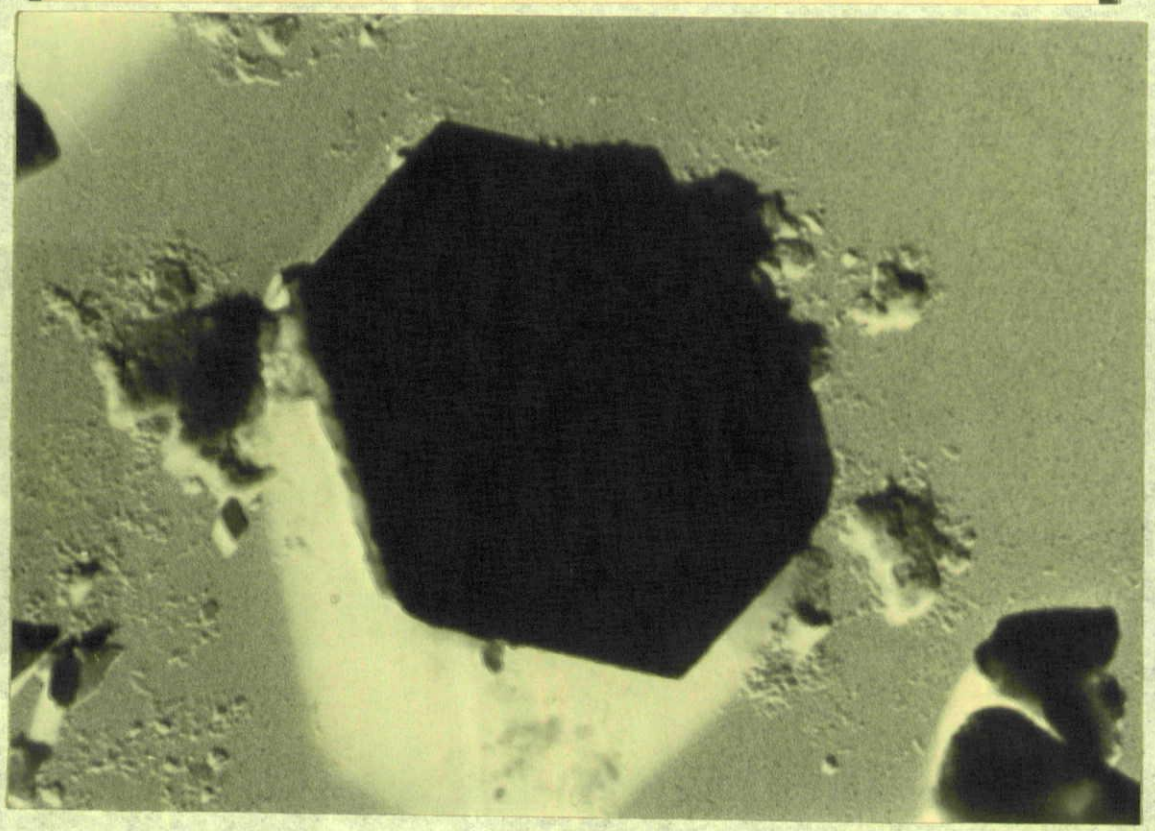
POLYGLYCINE I .- ex. glycine and water in phenol, heated at 140°C. for 4 weeks.



POLYGLYCINE I.- ex. glycine and hydrochloric acid polymerisation, Polyglycine II present as an impurity showing Moire fringes.

POLYGLYCINE II. ex. Calcium chloride solution after seven reprecipitations, showing thick and thin hexagons.



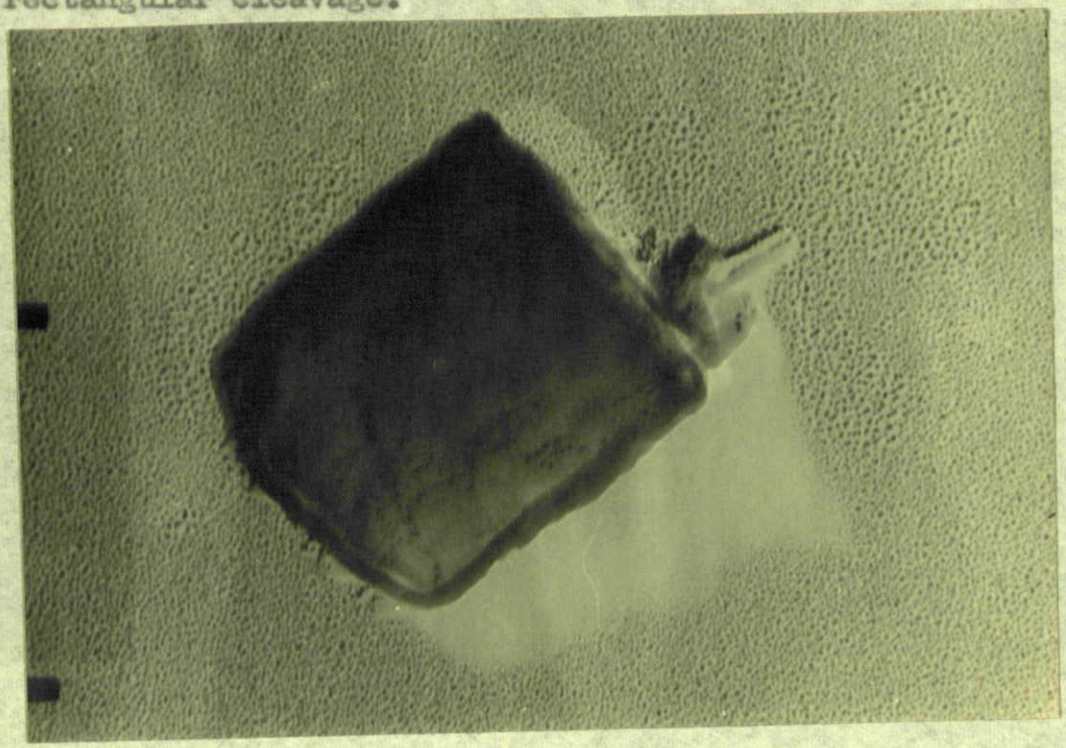


the expense of the thicker edges of the "donuts", which become of more even optical density when viewed in the electron beam. These rather thick near hexagons were the limit for the material, further reprecipitations produced no further change.

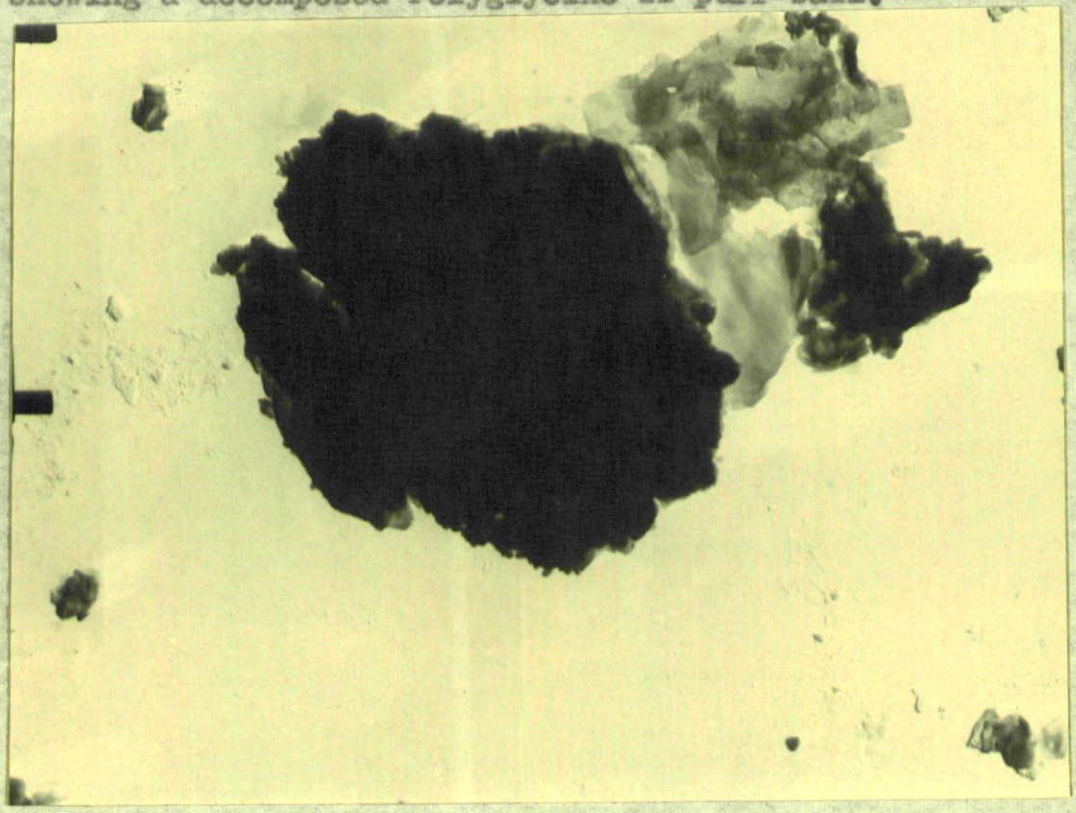
Precipitation from zinc chloride solution resulted in products which reached the thick "donut"stage, and finally stopped at the thick "donut" with rounded corners stage. The precipiations from calcium chloride solution produced the better thinner edged hexagons.

Polyglycine I obtained from the polymerisation of glycine and hydrochloric acid showed considerable quantities of the "puff-balls" and "donuts", showing that it contained some Polyglycine II. The remainder of the material was very small irregular shaped pieces. The Polyglycine I obtained from the polymerisation of glycine in phenol, showed a completely different form. No "puff-balls" or "donuts" were present. The material consisted of extremely thin strongly striated laminae, showing rectangular cleavage. Most of this material was of small size. There were present one or two large flat laminae, per whole grid, which were probably Polyglycine II, some showed a hexagonal shape and characteristic growth steps. Also much of the

POLYGLYCINE I. ex. glycine and phenol/water, showing rectangular cleavage.



POLYGLYCINE I. ex. glycine and hydrochloric acid, showing a decomposed Polyglycine II puff-ball.



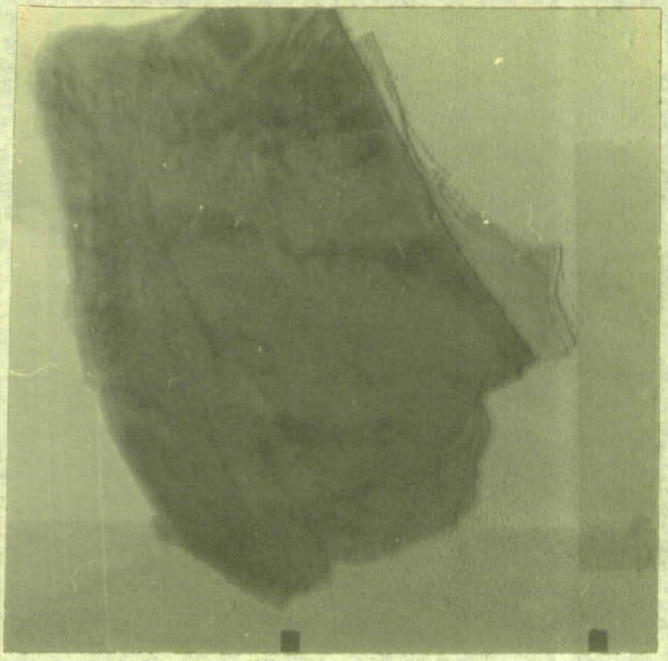
small material was clumped together. This occurred too often to be fortuitous, and it was assumed that these clumps were the remains of Polyglycine II "puff-balls" or "donuts". Thus agreeing with the other experimental work showing that Polyglycine II was first formed, which was then converted into Polyglycine I. The size of the clumps was about the same as that of the "puff-balls." The fact that this breakdown of the Polyglycine II structure was so drastic indicated that the structures of Polyglycine II and Polyglycine I were widely different. The Polyglycine I obtained from the polymerisation of piperazine-2,5-dione, diketopiperazine in water produced a similar product to that of the polymer from glycine and phenol. Very little Polyglycine II was present, as laminae, but the same decomposed puff balls appeared.

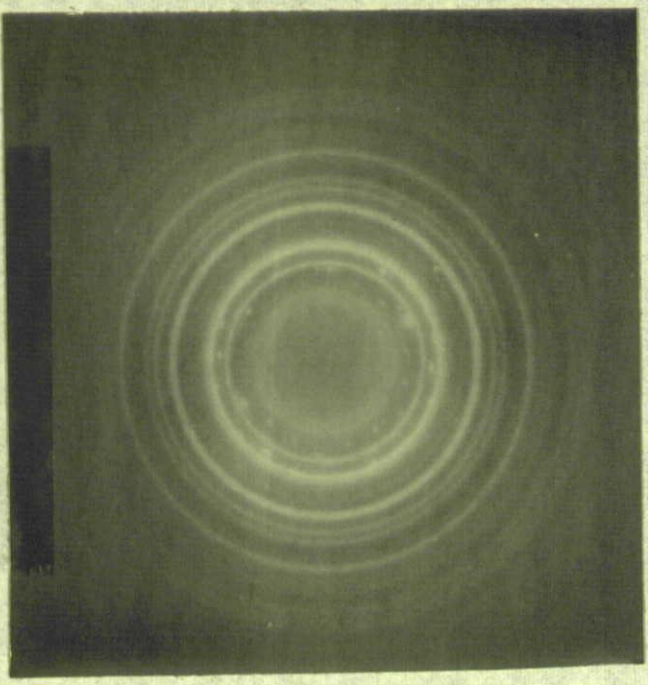
ELECTRON DIFFRACTION.-

It was not found possible to obtain electron diffraction patterns from the thick hexagons of Polyglycine II. Many of the samples were tried without any success. Even the thin edges and corners would not give a diffraction pattern. Thus it seemed that the crystals were in the wrong orientation to produce a diffraction pattern, and it was not just a question of specimen thickness. One very thin lamina, which showed Boire fringes, did produce a

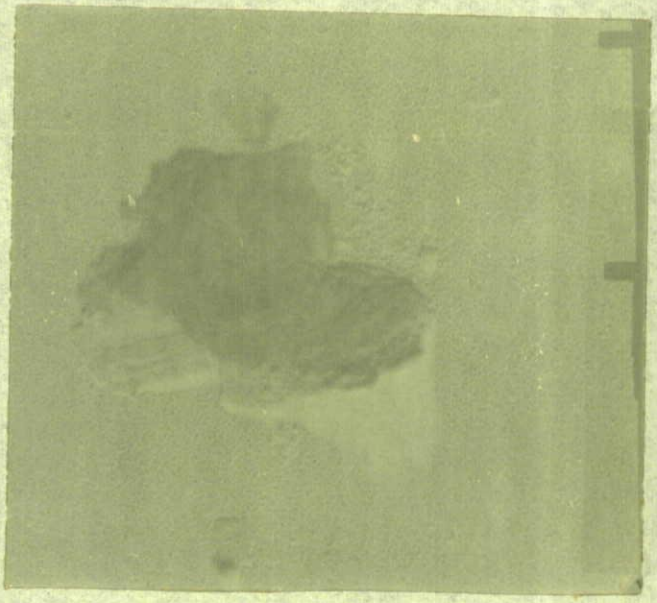
ELECTRON DIFFRACTION.-

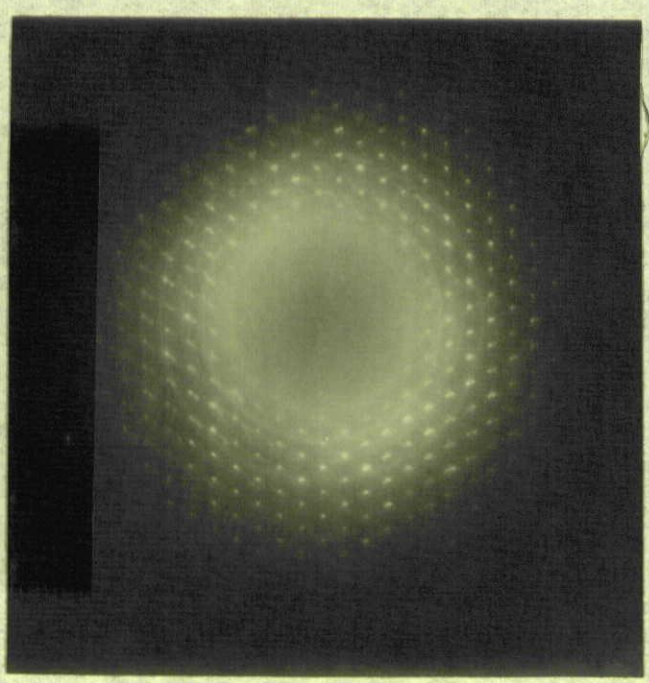
POLYGLYCINE I. ex. glycine and water in phenol, 140°C. 4 weeks, heated sealed. Sample and diffraction pattern. (probably due to Polyglycine II impurity)





POLYGLYCINE II. ex. calcium chloride solution, precipitated by water. Sample and diffraction photograph.



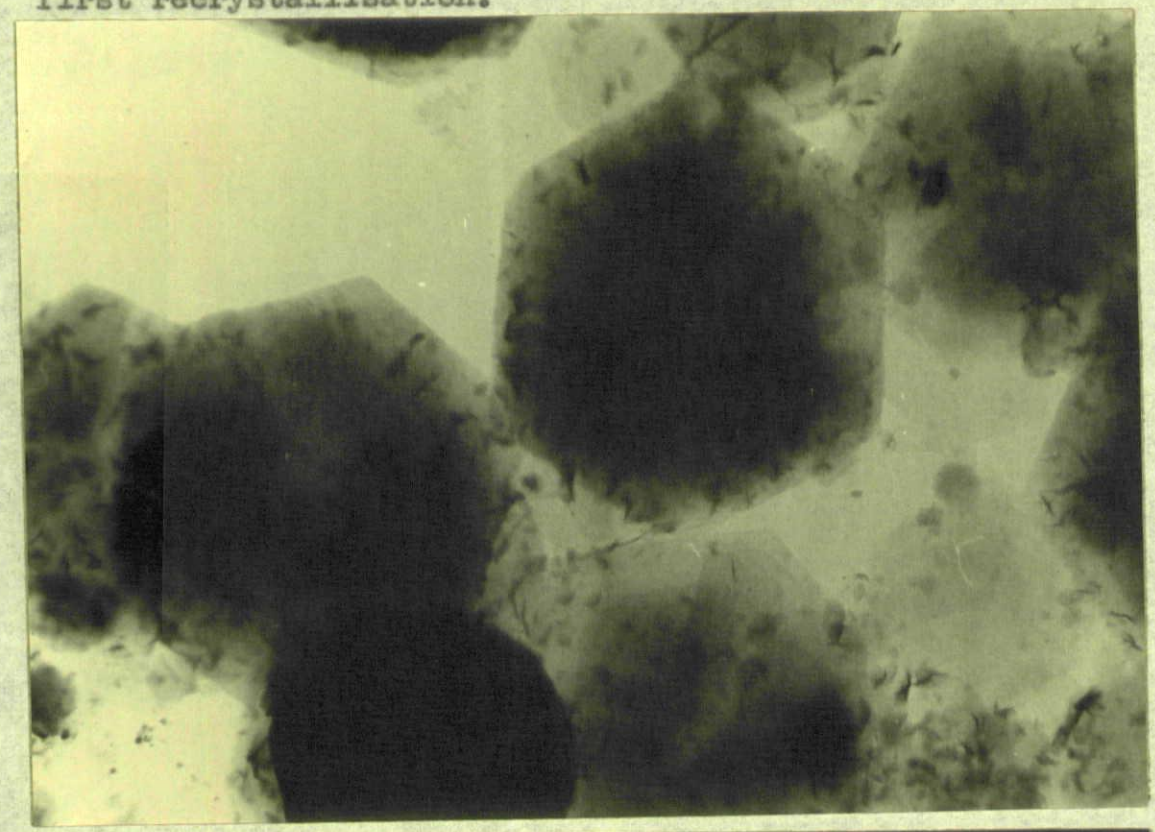


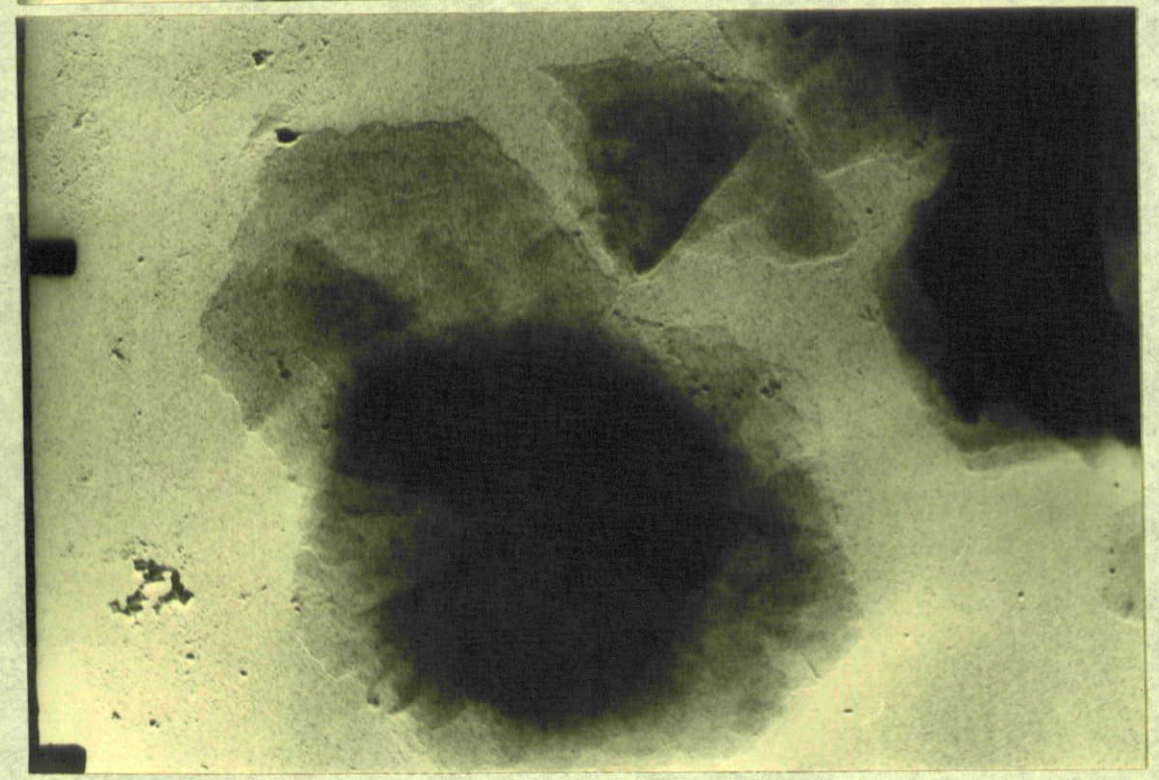
a diffraction photograph, this was a combined single crystal and powder type photograph. The rings were due to the gold shadowing metal. The remaining electron diffraction patterns were obtained only from irregular specimens, from which it was not possible to determine the specimen orientation. These patterns do however show an hexagonal arrangement of the spots.

Polyglycine I samples obtained by the different methods were also examined. The preceeding photographs showed that this material was far less well defined, as regards crystalline quality, than the Polyglycine II. The best formed sample was that derived from glycine and phenol/water polymerisation, it required one months heating. None of the thin striated laminae could be made to produce diffraction photographs. The photographs which were obtained were produced by the large flat laminae, which had shown Moire fringes. Hexagonal spot patterns were obtained. The patterns obtained and the reasons given above, led us to beleive that this material was Polyglycine II present as an impurity.

Little progress could be made with the electron diffraction samples since it was not possible to be sure of the orientation of the samples. It was also not easy to alter the orientation, except in one plane.

HOT WATER SOLUBLE POLYGLYCINE II.- ex. glycine and hydrochloric acid. Lower sample obtained from the first recrystallisation.



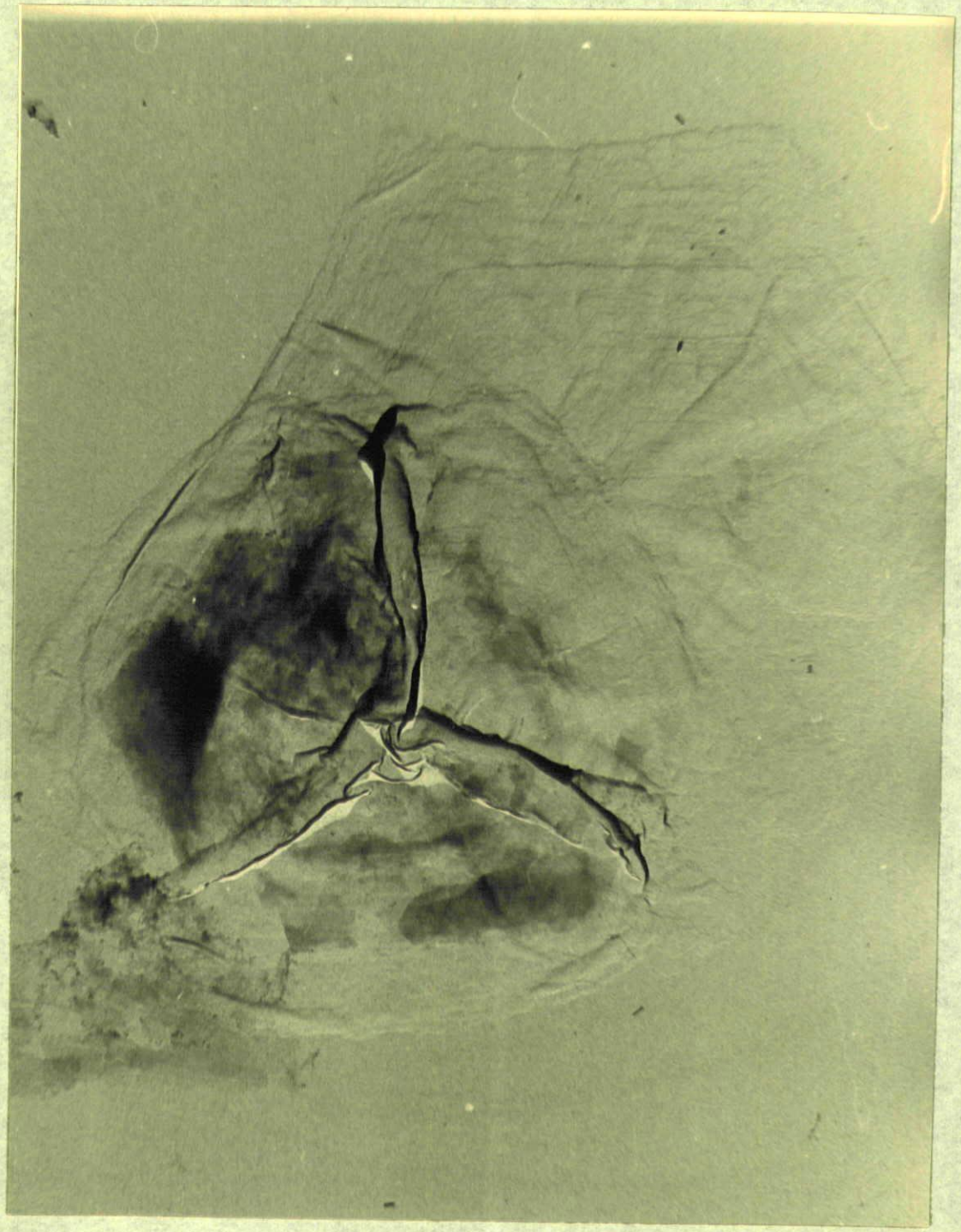


The homogeneity and the crystalline quality of both polymers would need improvement, especially in the case of Polyglycine I, before any serious results could be obtained.

WATER SOLUBLE PEPTIDE SHOWING THE POLYGLYCINE II STRUCTURE.-

The material obtained from the polymerisation of glycine in hydrochloric acid, was predominantly Polyglycine I. The examination under the electron microscope and the X-ray results showed that it also contained some Polyglycine II. IT was found that at least some of this Polyglycine II could be extracted by boiling the crude product with water, filtering, and allowing the filtrate to stand and cool. A "leafing" solid was deposited. This solid was left in the mother liquor, and drops placed on prepared carbon grids, and later clean mica strips. The examination of these grids under the electron microscope showed that the material consisted almost entirely of very thin hexagonal plates, or nearly hexagonal plates. These plates were so thin that the contrast between the specimen and the supporting film was so low that focussing was difficult. The photographic negatives obtained were also very difficult to print. The photographs do show distinct

HOT WATER SOLUBLE POLYGLYCINE II.- ex glycine and phosphoric acid, heated in the open. Product from the first recrystallisation from water.



step growth at the edges. It was found that unlike the other Polyglycine II specimens, the samples were very easily burnt up by the electron beam. The kiloveltage had to be reduced to 60 kv.. These specimens greatly resembled the specimens used by Keggy and Sikorski(1956)[91]. The product must be a water soluble, low molecular weight peptide, showing the Polyglycine II structure. This would caccount for the ease with which it could be burnt up in the elctron beam. This material was very similar to true Polyglycine II in its behaviour on attempted electron diffraction. No spot patterns could be obtained with the platelets lying in the plane of the grid at right angles to the electron beam.

A similar material of better crystalline quality was obtained from the polymerisation of glycine in phosphoric acid solution. The quantity of material obtained was much greater. The crystals were extremely thin hexagonal plates, and showed great detail of step growth and growth dislocations. This material is certainly the most promising material for a single crystal investigation of Polyglycine II. Again no electron diffraction photographs could be obtained with the platelets perpendicular to the electron beam direction.

THE X-RAY INVESTIGATION OF THE LOWER PEPTIDES OF GLYCINE.

POWDER PHOTOGRAPHY.

X-ray powder photographs were taken using both a nine centimetre Unicam powder camera, and a flat plate camera, with a 5 centimetre specimen to plate distance. The following peptides were examined.-

2,5-diketopiperazine,
glycylglycine hydrochloride monohydrate,
glycylglycine methyl ester hydrochloride,
glycylglycine ethyl ester hydrochloride,
diglycylglycine,
diglycylglycine methyl ester hydrochloride,
diglycylglycine ethyl ester hydrochloride,
triglycylglycine,
pentaglycylglycine,
pentaglycylglycine methyl ester hydrochloride.

Examination of the photographs showed that certain diffraction lines were common to more than one peptide photograph. Attempts were made to determine the structures of the polymers form these photographs, making use of the Ito method. This was not successful in a single case. The reason for this was thought to

be the extensive overlapping of the X-ray lines of any one polymer, and the systematic absences, due to glide planes or screw axes, which these lower symmetry structures may be expected to exhibit.

X-RAY DIFFRACTOMETRY.-

It was thought that the X-ray powder photographs obtained above could have been improved upon by the use of a camera of greater resolving power. The use of the X-ray diffractometry method immediately suggested itself. A Solus-Schall diffractometer was used; this is equivalent to a powder camera of twenty centimetres radius. The peak positions and relative intensities were recorded using an Elliott millivoltmeter type chart recorder[Note.- the pen of the recorder moves in an arc, this has to be taken into account when the line position is determined from the fiduciary marker. This fiduciary marker, marks at four degree intervals along the top edge of the chart.]. The specimens used were compressed pellets,3/16" diameter and 1/16" thick. The chart traces showed much more detail than the photographs and could be more readily compared. To the authors knowledge there is no published data on the X-ray powder lines of these lower peptides, and as full a set of data as possible has been included

in this work and it is hoped that it will be of use to future workers. The X-ray diffractometer traces, contact copies of the original traces, are shown in Charts 12 and 13 at the end of the thesis.

The methods used for the preparation of the specimens and the results obtained are shown below.

1) GLYCINE.

The sample used was B.D.H. analar quality glycine. The diffractometer trace was included for comparison purposes only, in order to show its presence in other preparations.

2) DIKETOPIPERAZINE.

This was prepared from analar glycine, by heating in glycerol, according to the method of Schott, Larkin, Rockland and Dunn[104]. This trace has also been included as a comparison trace.

3) GLYCINE HYDROCHLORIDE.

This was prepared from analar glycine by the method of Frost[60]. This sample was prepared as an intermediate in the preparation of diglycine hydrochloride. The diffractometer trace was used to show that the diglycine hydrochloride was pure.

4) GLYCINE ETHYL ESTER HYDROCHLORIDE.

The sample was prepared by the method of Greenstein and Winitz[63]. The melting point of the product was 145°C. The accepted value is 145-148°C.. A chromatogram showed that it contained traces of glycine but no glycine lines were found in the diffractometer trace. This trace was again used for comparison only.

5) DIGLYCINE HYDROCHLORIDE.

The preparation was carried out according to the method of Frost[60], the melting point of the product was 186°C. agreed with this workers value. This material is not to be confused with glycylglycine hydrochloride, it is formed by the crystallisation together of glycine and glycine hydrochloride. The diffractometer trace was used for comparison with that obtained from a hydrolysed sample of glycylglycine ethyl ester hydrochloride, see Appendix I. The crystal structure of this compound has been studied in detail by Buerger and Hahn[30].

6) GLYCYLGLYCINE HYDROCHLORIDE MONOHYDRATE.

This was prepaered by the partial acid hydrolysis of diketopiperazine with concentrated hydrochloric acid, by the method of Fischer and Fourneau[51]. The diffractometer trace was used to determine the

purity of glycylglycine ethyl ester hydrochloride, and to see which X-ray lines, if any, could be attributed to the glycylglycine backbone. Work has also been carried out on the crystal structure of glycylglycine, by Bernal[26], who distinguished three different crystalline modifications, the α, β, and γ forms. The structure of the β-form has been determined by Hughes and Moore[70], and they are still working on the α-form structure. The structure of glycylglycine hydrochloride monohydrate does not resemble any of the three accepted structures for glycylglycine. There is however a distinct agreement in line position, with three lines in the pattern for glycylglycine ethyl ester hydrochloride. The peaks which are common to both patterns are.-

θ°	$G_2.HCl.H_2O$		$G_2Et.HCl.$		d/n A.
12°	8'	(s)	12°	6' (m)	3.67
13°	18'	(s)	13°	15' (v.s.)	3.35
15°	45'	(w)	15°	45' (m)	2.84

7) GLYCYLGLYCINE ETHYL ESTER HYDROCHLORIDE.

This specimen was prepared by the esterification of some of the glycylglycine hydrochloride monohydrate prepared above. The method used for the preparation was that of Schott, Larkin, Rockland and Dunn[104].

The specimen had a melting point of 185-186°C., this agreed well with the value obtained by the above workers. The product was also chromatographically pure. Comparison of the Diffractometer trace obtained with that of glycylglycine hydrochloride monohydrate, showed that none of this material was present as an impurity. This comparison did show that the structures were somewhat similar, in that they have some lines in common. These common lines were at first thought to be due to the ionic hydrochlotide lattice, but further investigation showed that no such lines appeared in the ester hydrochlorides of diglycylglycine. Thus they are probably a function of the glycylglycine group in these compounds. It is unlikely that the peptide chain would take control of the structure as early as the tripeptide stage. This is borne out by the X-ray pattern for this peptide being totally dissimilar to the true polypeptide X-ray pattern.

8) GLYCYLGLYCINE METHYL ESTER HYDROCHLORIDE.

The author was not able to find any published data on this compound. Several methods of preparation were attempted, all attempts were made using glycylglycine hydrochloride monohydrate as the starting material.

Method 1.- 30 gm.(0.25 moles) of glycylglycine hydrochloride monohydrate was boiled with 200 ml. of methanol, made one normal with respect to hydrochloric acid, until all the crystals had dissolved. The methanol was evaporated off in vacuo until the volume had been reduced to 50 ml.. Dry ether was added to precipitate the product. The crystals obtained were filtered off at the pump, and washed with ether. The crystals were recrystallised from 20 ml. of methyl alcohol, the yield was 18 gm., the melting point was 129°C.. Variations of this method using different proportions of hydrochloric acid were tried. The product in each case had a melting point of 129±1°C.. A phase change appeared to occur on melting, the sample appeared to shrink, evolving water, started to melt, changed to smaller crystals and then melted. The X-ray pattern of this material showed strong reflections of all the prominent lines of glycylglycine hydrochloride monohydrate. There were no other strong reflections present. This suggested that the sample was only partially methylated. The remaining weak reflections which could not be attributed to the hydrochloride monohydrate are due to glycylglycine methyl ester hydrochloride. A chromatogram(butanol,pyridine,water;1:1:1 upper phase) of the product confirmed the presence of glycylglycine

hydrochloride hydrate, it ~~is~~ also showed a faster moving spot having a similar R_f value to that for glycylglycine ethyl ester hydrochloride.

Method 2.- The method used above for the preparation of glycylglycine ethyl ester hydrochloride was tried, this again gave a mixed hydrochloride hydrate and ester hydrochloride product, with the same melting point.

The X-ray reflections assumed due to the methyl ester hydrochloride do show a superficial resemblence to reflections for the ethyl ester hydrochloride. This would be expected since the structures must be similar. The reflections are however too weak to draw any definite conclusions.

There is no obvious reason why the methyl ester hydrochloride should be difficult to prepare in a pure state, unless it is much more easily hydrolysed than the corresponding ethyl ester hydrochloride.

9) DIGLYCYLGLYCINE.

This was prepared by the ammonolysis of chloracetyl glycylglycine according to the method of Abderhalden[1]. The chloracetyl glycylglycine was prepared from diketopiperazine by the method of Fischer[50]. This method also produces traces of other higher peptides. These were removed by several

recrystallisations from hot aqueous ethanol. The higher peptides were insoluble in this medium . The product had a melting point of 246°C. with decomposition. A chromatogram(butanol, pyridine and water,1:1:1, upper phase), showed that the product was chromatographically pure. Slow recrystallisation from aqueous ethanol, in a desiccator over calcium chloride produced good single crystals. A single crystal X-ray examination of these crystals was not attempted, since this material had been previously investigated by Bernal[26], The method of preparation was the same as that used for this investigation. Bernal also stated that no other crystalline modifications were observed. The X-ray powder diffractometer trace was therefore merely taken as a means of comparison with those of the corresponding methyl and ethyl ester hydrochlorides. It has become apparant during the writing up of this work that the material obtained did not have a structure corresponding to that proposed by Bernal. The Bernal cell has the following dimensions.-

$a = 22.0$ A., $b = 9.8$ A., $c = 4.7$ A., $\beta = 90°$.

Thus the unit cell belongs to the orthorhombic system, and contains four molecules. The composition of the material is given as diglycylglycine dihydrate

31	20°	48'	2.18 A.	v.w.
32	21	6	2.14	m. 5th.order of 1).
33	21	30	2.10	v.v.w.
34	22	12	2.04	v.v.w.2nd. order of 9).
35	23	3	1.97	w. 5th. order of 2).
36	24	6	1.89	v.w. 2nd. order of 11).
37	24	27	1.86	v.w.
38	25	3	1.82	v.v.w. {4th. order of 4). 5th. order of 3).
39	25	34	1.79	v.w. {2nd. order of 12). 6th. order of 1).
40	25	54	1.75	v.w.
41	27	42	1.66	v.w. } d 6th. order of 2).
42	28	6	1.64	v.w. }
43	28	30	1.62	v.v.w.
44	29	57	1.54	v.v.w. 7th. order of 1).

key.- d signifies a doublet, sh. signifies a shoulder

BERNAL CELL

HKL	θ°_{hkl}		d/n A.	d/n A.(observed).
100	2°	0'	22.0	absent.
010	4	30	9.8	9.94
001	9	24	4.7	4.79
200	4	30	9.8	10.77
020	9	2	4.9	4.93
002	19	8	2.35	2.35
110	4	54	9.0	9.10
011	10	30	4.23	4.08
101	9	39	4.60	4.40
111	10	34	4.18	4.08

DIGLYCYLGLYCINE X-RAY PATTERN.

No.	Bragg θ°	d/n A.	strength	comment.
1	4° 6'	10.77	v.v.w.	
2	4° 27'	9.94	m.	
3.	4. 51	9.10	v.s.	
4	6 3	7.30	w.	
5	8 12	5.40	w.	2nd. order of 1)
6	9 0	4.93	v.v.w. } d	2nd. order of 2)
7	9 15	4.79	v.w.w }	
8.	10 3	4.40	v.s.	
9	10 50	4.08	v.s.	
10	11 3	4.00	v.s.	
11	12 5	3.68	m	2nd. order of 4)
12	12 24	3.59	m.	3rd. order of 1)
14	13 5	3.40	v.s.	
14	13 24	3.32	w. sh.	3rd. order of 2)
15	13 39	3.25	w. sh.	
16	14 12	3.14	w } d	
17	14 26	3.09	w. }	
18	14 39	3.05	m.	
19	15 0	2.98	w.	
20	15 12	2.94	v.w.w	
21	15 24	2.90	m.	
22	16 34	2.70	v.s.	2nd. order of 5) 4th. order of 1)
23	17 9	2.61	m.	
24	18 9	2.48	m.	4th. order of 2)
25	18 33	2.42	v.w. } d	3rd. order of 4)
26	18 45	2.39	w. }	
27	19 6	2.38	v.w.	
28	19 18	2.35	v.w.	
29	19 39	2.29	v.w.	
30	20 9	2.22	v.v.w.	

[$G_3.2H_2O.$]. Calculation of several values of hkl for this cell showed differences between the observed and the calculated X-ray spacings of 1 to 10%. The calculated density(1.486 gm./ml.) for this cell, was about 1% low compared with the observed value of 1.512 gm./ml.. This is unusual since the calculated density is usually high compared with the observed value. The errors in the spacings are too large to be accounted for as experimental error, also the structure does not account for a very strong spacing observed at 11° 3'(4.00 A.). A similar trace using a chart speed of 30" per hour showed no trace of a peak at 22.0 A.. The first peak appeared at 4° 6'(10.77 A.). It may be that the a-axis is a screw diad since none of the odd reflections for h00 appear except as possible weak reflections, whereas the even orders appear down to h = 14. Some of the polymer was melted to see if water of crystallisation was evolved; no condensation was observed in the cold part of the capillary. There can be no doubt that this material is diglycylglycine and that it is a different crystalline form from that observed by Bernal. It is probable that this form is not hydrated.

DIGLYCYLGLYCINE ETHYL ESTER HYDROCHLORIDE X-RAY TRACE.

No.	Bragg θ°	d/n A.	strength	Comment.
1	2° 48'	15.79	v.s.	
2	7 42	5.75	v.s.	
3	7 57	5.55	v.v.w.	
4	8 15	5.38	v.v.w.	
5	8 36	5.16	w.	
6	9 3	4.90	v.v.w.	
7	10 4	4.40	v.w.	
8	10 24	4.25	v.w.	
9	11 30	3.87	v.s.	
10	12 0	3.71	v.w.	
11	12 21	3.60	s.	diglycylglycine (m). (common peak.)
12	12 33	3.55	w.	
13	13 18	3.35	w.	
14	14 6	3.16	v.w.	5th. order of 1).
15	14 30	3.08	m.	
16	14 48	3.02	s.	
17	15 15	2.93	w.	2nd. order of 8).
18	16 4	2.78	w.	2nd. order of 3).
19	17 30	2.56	m.	
20	18 6	2.48	v.v.w.	
21	18 18	2.45	v.v.w.	
22	18 51	2.38	w.	
23	19 36	2.28	v.v.w.	7th. order of 1)?
24	20 15	2.23	w. } d	
25	20 24	2.21	w. }	
26	21 4	2.14	v.v.w.	
27	21 45	2.08	m.	compare with Polyglycine II.
28	22 9	2.04	v.v.w.	
29	22 54	1.98	v.v.w.	

No.	Bragg θ°		d/n A.	strength	Comment.
30	23°	30'	1.93	v.w.	(2nd. order of 9).
31	24	3	1.89	v.v.w.	(3rd. order of 2).
32	24	42	1.84	v.v.w.	3rd. order of 3).
33	25	6	1.81	band.	
34	25	48	1.77	v.v.w.	
35	26	18	1.74	v.v.w.	
36	27	15	1.68	v.v.w.	
37	27	45	1.65	v.w.	
38	29	18	1.57	v.w.	
39	30	24	1.52	v.v.w.	
40	32	36	1.43	v.v.w.	
41	33	9	1.41	v.v.w.	
42	34	0	1.38	v.v.w.	
43	36	15	1.30	v.v.w.	

d = doublet

This is probably a new form of this peptide; time did not allow a fuller investigation.

10) DIGLYCYLGLYCINE ETHYL ESTER HYDROCHLORIDE.

This compound was prepared from diglycylglycine as obtained above. 5 gm. of diglycylglycine was refluxed together with 5 ml. of concentrated hydrochloric acid and 150 ml. of absolute ethanol. A little of the peptide remained undissolved, this was filtered off and the clear solution evaporated down under vacuum, to about half its volume. The residue in the flask was then treated with 15 ml. of ether and allowed to stand overnight. The crystals which had deposited were filtered off at the pump, and washed several times with ether. The yield was 5 gm., the melting point of the product was 216°C. This agreed very well with the value obtained by Rydon and Smith[101]. A chromatogram using butanol, pyridine and water(1:1:1; upper phase) as the moving phase, showed that the material was pure. The X-ray powder diffraction trace certainly showed no lines due to diglycylglycine. Strong peaks occurred on the diffractometer trace at low Bragg angles. A very strong peak occurred at 2° 48' (15.79 A.) but only two very weak possible higher order peaks, the fifth and the seventh appeared. Some of the peaks showed second and third order

DIGLYCYLGLYCINE METHYL ESTER HYDROCHLORIDE X-RAY TRACES.

No.	Bragg θ°		d/n A.	strength	Comment.
1	3°	3'	14.5	v.s.	
2	4	48	9.20	v.v.w.	
3	6	3	7.30	v.s.	2nd. order of1).
4	7	6	6.24	v.v.w.	
5	8	24	5.27	v.v.w.	
6	9	12	4.82	w.	3rd. order of 1).
7	9	45	4.57	v.w.	2nd. order of 2).
8	10	24	4.25	w.	
9	11	12	3.95	v.w.	
10	11	36	3.82	v.w.	
11	12	4	3.67	v.w.	
12	12	42	3.50	w.	
13	12	54	3.45	w.	
14	13	33	3.21	w.	{2nd. order of 4)? {3rd. order of 2).
15	14	30	3.08	v.w.	
16	15	33	2.88	v.w.	5th. order of 1).
17	16	18	2.74	v.w.	
18	17	30	2.565	v.w. band	
19	18	0	2.496		
20	19	12	2.34	v.v.w.	
21	19	30	2.31	w.	
22	20	42	2.18	v.v.w.	
23	21	36	2.09	v.v.w.	{7th. order of 1). {3rd. order of 4).
24	21	57	2.06	v.v.w.	d compare Polyglycine II (200) 2.08 A.
25	23	0	1.98	v.v.w. band	
26	23	45	1.91	v.v.w.	
27	24	30	1.86	v.v.w.	
28	26	33	1.72	v.v.w.	
29	27	45	1.65	v.v.w.	
30	28	12	1.63	v.v.w.	
31	30	3	1.54	v.v.w.	
32	35	30	1.33	v.v.w.	

reflections but no other higher order reflections appeared. Attempts have been made to determine a unit cell for this compound but without any success. An attempt to grow single crystals from a dilute solution in aqueous ethanol produced good crystals, but analysis showed that these were about 80% or less of the ester hydrochloride, and that hydrolysis had occurred in solution.

11) DIGLYCYLGLYCINE METHYL ESTER HYDROCHLORIDE.

This compound was prepared in the same way as the previous compound, except that the ester hydrochloride crystallised out on standing without the addition of ether. The crystals obtained were nearly rectangular thin plates. The yield from 4 gm. of peptide was 3 gm. of ester hydrochloride, the melting point of the product was 196°C.(Rydon and Smith quoted a value of 197°C.). A chromatogram showed this to be free from tripeptide. The X-ray diffractometer trace again showed very strong low angle reflections, in this case a reflection appeared at 3° 3'(14.5A.). This reflection is repeated down to the seventh order, except for the sixth order, which is absent. In fact the first and second order peaks for this reflection are the only very strong peaks shown. It is peculiar that a reflection

TRIGLYCYLGLYCINE X-RAY TRACE.

No.	Bragg θ°	d/n A.	strength	Comment
1	9° 12'	4.82	v.v.w.	
2	9 56	4.45	m.	
3	10 30	4.23	m.	
4	11 30	3.87	w.sh.	
5	11 51	3.73	w.sh.	
6	12 3	3.69	w.p.	
7	12 9	3.66	w.	
8	12 27	3.57	w.	
9	12 48	3.48	v.s.	
10	13 21	3.34	v.s.	G/H_3PO_4 2nd. wash
11	15 0	2.98	w.	ditto. ($13^{\circ}24'$, v.s.).
12	15 39	2.86	v.v.w.	
13	16 12	2.76	v.w.	
14	16 54	2.65	v.v.w.	
15	17 6	2.62	v.v.w.	
16	17 30	2.56	v.v.w.	
17	17 45	2.52	v.v.w.	
18	18 21	2.44	v.v.w.	
19	19 33	2.30	w.	
20	19 57	2.26	w½	2nd. order of 2).
21	20 42	2.18	w.p.	
22	20 54	2.16	w.sh.	
23	21 24	2.11	w.	2nd. order of 3).
24	21 54	2.06	v.w.	
25	21 59	2.05	v.w.	
26	22 24	2.02	v.w.	
27	23 6	1.96	v.w.	
28	23 57	1.90	v.w.	
29	25 15	1.81	v.w. band	
30	27 0	1.70	v.w.	
31	27 6	1.69	v.w.p.	
32	28 30	1.62	v.w. band.	

occurs at 2.09 A., a similar reflection at 2.08 A. is suggested as the 200 reflection for the Crick structure for Polyglycine II. A similar reflection also occurs in the diffractometer trace recorded for diglycylglycine ethyl ester hydrochloride. No such reflection occurs in the trace recorded for diglycylglycine.

12) TRIGLYCYLGLYCINE.

This sample was prepared by the Fischer method. Diglycylglycine was treated with chloracetyl chloride, to form the chloracetyl compound. Ammonolysis of the chloracetyl compound gave triglycylglycine. The material obtained was a white microcrystalline powder. The material darkened when heated to 220°C., and melted at 262°C., this agreed well with the value given in Beilstein. This peptide is only sparingly soluble in cold water, but it is soluble in hot water. A chromatogram(butanol,pyridine, and water) showed that the material was pure. The X-ray diffractometer trace showed four prominent lines, and two less prominent ones. No low angle peaks occur. The prominent peaks occur at 9° 56'(4.45A.)(m), 10°30'(4.23A.)(m), 12°48'(3.48A.)v.s., 13°21'(3.34A.) (v.s.). The 13°21' peak is also shown by the material obtained from waterwashing of the product from the

PENTAGLYCYLGLYCINE X-RAY PATTERN.

No.	Bragg θ°	d/n A.	strength	Comment.
1	2° 0'	22.10	w.sh.	
2	4 12	10.52	v.w.	
3	6 45	6.56	v.w.	
4	7 21	6.04	v.w.	
5	8 18	5.34	v.w.	8° 21' peak present in $G_6Me.HCl$.
6	9 33	4.64	v.w.sh.	
7	10 6	4.38	w.sh.	
8	10 31	4.20	v.s.	10°38'Polyglycine II $G_6Me.HCl$.
9	11 4	3.99	v.w.	
10	11 30	3.87	w.	
11	11 54	3.72	w.	3.78A. Polyglycine II.
12	12 27	3.57	v.w.	
13	13 0	3.43	v.w.	
14	13 45	3.24	w.	
15	14 0	3.19	v.w.	
16	14 24	3.10	v.s.	14°24'Polyglycine II.
17	15 9	2.95	w.	
18	16 3	2.75	v.w.	
19	16 54	2.65	w.	
20	17 45	2.53	w.	
21	18 45	2.39	w.	18°43'Polyglycine II.
22	19 24	2.32	v.w.	19°24'Polyglycine II.
23	20 48	2.17	v.w.	
24	21 21	2.12	v.w.	
25	21 45	2.08	v.w.	2.08A.Polyglycine II.

sh = shoulder.

Note.- This diffraction pattern is dealt with more fully under a comparison with that of Polyglycine II, and more accurate results are shown there. See Sec.17..

polymerisation of glycine in phosphoric acid (see Exp. 8.). There are however no other X-ray peaks held in common by both these compounds. There are only first and second order peaks present in the trace, no higher order peaks occurred. There is no 2.08 A. peak, and the pattern differs entirely from those of the higher peptides. The pattern does not bear any resemblences to any of the lower peptides either.

13) TETRAGLYCYLGLYCINE.

There was insufficient time to prepare samples of this peptide, or any of its esters.

14) PENTAGLYCYLGLYCINE.

This sample was prepared by the method of Fischer[50]. 4.8 gm. of diglycylglycine methyl ester hydrochloride was dissolved in 150 ml. of hot dry methanol, and 30 ml. of a solution of 3 gm. of sodium in 200 ml. of absolute methanol were added. The methanol was then distilled off on a boiling water bath, 30 ml. of water and 10 ml. of 2N sodium hydroxide solution were mixed and added to the dry residue; this mixture was heated on a water bath for 5 minutes to hydrolyse the ester. 10 ml. of 2N acetic acid and 250 ml. of water were added and the mixture boiled and filtered. The very slight residue

could not be removed from the filter paper. The filtrate was allowed to stand overnight in a refrigerator, a white microcrystalline deposit was obtained. The yield was 1.7 gm.(47%). The crystals were dissolved in 500 ml. of boiling water, and the solution cooled and filtered. This recrystallised material was shown to be chromatographically pure, running as a single spot with no tailing. The results obtained from the titration of the carboxyl end groups in formaldehyde solution, using sodium hydroxide and phenolphthalein confirmed that this compound was a hexapeptide. The X-ray diffractometer trace for this material showed a strong similarity to that for Polyglycine II. Strong peaks occurred at 10° 31' and 14° 24', corresponding to spacings at 4.20 A, and 3.10 A. respectively. The corresponding values for Polyglycine II are 10° 38'(4.15A.) and 14° 24'(3.10 A.). The latter peak is of much lower intensity in the Polyglycine II trace than in that for pentaglycylglycine. It is peculiar that the former peak has a lower value for the angle θ in this case, but this low value is also shown by the corresponding line in the trace for benzoyl pentaglycylglycine, but is not shown by the low molecular weight peptides obtained from the

PENTAGLYCYLGLYCINE METHYL ESTER HYDROCHLORIDE.

X-RAY TRACE.

No.	Bragg θ°	d/n A.	strength	Comment.
1	2° 43'	16.25	m.	
2	5 18	8.34	w.	
3	7 12	6.15	v.w.	
4	7 57	5.55	w.	
5	8 21	5.31	v.w.	$8^{\circ}18'G_6$-Peptide.
6	9 27	4.69	v.w.sh.	$9^{\circ}27'G_6$-Peptide.
7	9 51	4.49	v.s.	
8	10 21	4.34	m.sh.	
9	10 31	4.20	m.p.	10° 38' PolyglycineII.
10	10 45	4.11	m.p.	
11	10 57	4.03	v.w.sh.	
12	11 12	3.95	v.w.sh.	
13	11 24	3.88	w.p.	
14	11 54	3.72	v.s.p.	
15	12 54	3.45	w.	
16	13 12	3.37	v.s.	
17	14 10	3.15	w.	
18	14 24	3.10	m.	$14^{\circ}24'$Polyglycine II.
19	15 8	2.96	v.w.	
20	15 30	2.89	v.w.	
21	15 57	2.81	v.w.	
22	16 33	2.70	v.w.	
23	17 12	2.61	v.w.	
24	17 48	2.52	v.w.	$17^{\circ}45'G_6$-Peptide.
25	18 10	2.47	w.	
26	19 3	2.36	v.w.	
27	19 36	2.29	v.w.	

No.	Bragg θ°	d/n A.	strength	Comment.
28	20° 30'	2.61	v.w.	
29	21 6	2.14	v.w.	
30	21 36	2.09	v.w.	
31	23 21	1.94	v.w.	
32	25 39	1.78	v.w.	
33	28 48	1.60	v.w.	
34	29 45	1.55	v.w.	
35	29 57	1.54	v.w.	

polymerisation of glycine in phosphoric acid. This difference is however only just beyond the limit of experimental error, approximately 6'of arc. Thus the structure of pentaglycylglycine closely resembles that of Polyglycine II, see Exp. 16..

15) PENTAGLYCYLGLYCINE METHYL ESTER HYDROCHLORIDE.

Crude pentaglycylglycine methyl ester hydrochloride, was prepared by the first part of the method used for the pentaglycylglycine polymerisation. The crude hexapeptide ester in solution in methanol, is decanted from sodium chloride and evaporated to dryness in vacuo at 40°C. The solid residue is extracted with chloroform, the residue remaining is hexapeptide methyl ester hydrochloride, and higher peptide esters. This residue is then extracted with boiling water, and the insoluble higher peptides filtered off, at the pump. The filtrate was then cooled and treated with a little ether and allowed to stand overnight. A deposit of white amorphous powder was obtained. The yield was 58%. The material melts with decomposition at about 240°C. A chromatogram showed that the product contained some hexapeptide. The X-ray diffractometer trace confirmed this, showing low intensity peaks for the hexapeptide. The peaks of the hexapeptide methyl ester hydrochloride

are easily distinguished from the hexapeptide peaks, by comparison of the two traces. The hexapeptide ester hydrochloride shows strong peaks at 2° 43'(16.25A.)(m), 9° 51'(4.49A.)(v.s.), 10° 45' (4.11A.)(m), 11° 54'(3.72A.)(v.s.), 13° 12'(3.37)(v.s.). It is interesting that this material shows a long spacing, not shown by the hexapeptide, see Exp. 17.. This is the 2° 43'(16.25A.)(m) reflection, this is more intense than the low angle spacings shown by pentaglycylglycine.

THE X-RAY EXAMINATION OF THE POLYGLYCINES.

The results obtained in this section were taken from diffractometer traces run at both three inches per hour chart speed, and thirty inches per hour. The accuracy of the results is better on the long faster chart traces, for the stronger more easily distinguished peaks, than on the shorter slow traces. For the weaker peaks, the accuracy was not improved by using the faster chart speed. The peaks were much less easily distinguished from the background radiation trace, and their maxima much less easily determined. This can be seen by comparison of the long and the short charts shown for the sample of recrystallised Polyglycine II.

POLYGLYCINE I.

It has been shown in the experimental section that it was extremely difficult to obtain pure samples of Polyglycine I, free of Polyglycine II. It was even more difficult to obtain well crystallised specimens of Polyglycine I. The best specimen was obtained by the polymerisation of diketopiperazine in water(see Exp. Sec. 7.,p.5.). This sample showed only a very weak peak at 10° 37'(4.15A.) corresponding to the very strong peak of Polyglycine II. Thus it can be safely assumed that all the other peaks are due to Polyglycine I only. The peaks are

POLYGLYCINE I X-RAY DIFFRACTION TRACE (3"per hour.)

No.	Bragg θ°	d/n A.	strength	Comment.
1	9° 9'	4.58	v.w.	
2	10 9'	4.36	v.s.	Astbury's 4.4A.(10° 3') interplanar spacing.
3	10 45	4.11	w.	Polyglycine II(10°37')
4	11 54	3.57	w.	
5	13 6	3.40	v.s.	Astbury's 3.45A.(12°55'). side chain spacing
6	14 18	3.12	w.p.	Not due to Polyglycine II
7	14 42	3.03	w.sh.	
8	14 51	3.01	v.w.sh.	
9	15 28	2.89	v.w.sh.	
10	16 3	2.78	v.w.p.	
11	16 30	2.72	v.w.p.	
12	16 52	2.66	v.w.sh.	
13	17 45	2.53	v.w.sh.	
14	18 6	2.48	v.w.p.	not due to Polyglycine II intensity too high.
15	19 9	2.35	v.w.	
16	19 51	2.27	v.w.	possible 2nd. order of1)
17	20 7	2.24	v.w.	
18	20 39	2.18	w.	2nd. order of 2)(2.18A.).
19	21 0	2.15	w.	
20	21 30	2.10	w.	
21	21 48	2.07	w.	
22	22 9	2.04	v.w.sh.	2nd order of 3)(2.05A.).
23	23 15	1.96	v.w.p.	
24	24 57	1.83	v.w.sh.	
25	26 52	1.70	v.w.sh.	2nd. order of 5)(1.70A.).
26	27 12	1.69	v.w.p.	
27	27 40	1.66	v.w.sh.	
28 [	32 6	1.45	v.w. band]	
29	32 15	1.44	v.w.p.	2nd.order of 9)(1.45 A.)
[	34 24	1.36	v.w.band]	band width 2°18', 2nd. order of 11)(1.36A.).
30 [	40 48 42 18	1.16	v.w.band]	1.16A. reflection for 41°40',(006) quoted by Bamford. 2nd. order of 15)(1.17A.)(41° 6').

key to results shown on p. 2a.-

sh = shoulder attached to the peak marked with a p.

The peaks marked as being not due to Polyglycine II, are those in which Polyglycine I shows similar spacings. These peaks cannot possibly be due to Polyglycine II. since in comparison with the most intense Polyglycine II peak, these peaks are too intense.

The values of θ given for the shoulders are measured at the mid point of the shoulder.

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not at all well defined. The main strong peaks which have been observed previously by other workers are quite clear. The weaker peaks, some of which have not been previously observed, showed some splitting into separate peaks. On all the powder photographs they appeared as broad bands.

Consider first of all, the short three inches per hour chart for this Polyglycine I. The chart showed no distinct peaks at lower angles than $\theta = 9^{\circ}$(see Chart 8.). The very weak peak at $\theta = 9^{\circ}$ 9'is not very distinct either. The chart shown was only one of several run from the same polymer specimen; only those peaks which appeared on more than one chart are recorded in the results shown opposite this page. The strong peak at 10° 9' (4.36 A.) corresponds to the 10° 3'(4.4 A.) peak recorded by Bamford et alia(1955)[17] and Astbury et alia(1948,1949)[9,8]. The very strong peak at 13° 6'(3.40A.) also agreed with the peak recorded by the above workers at 12° 55'(3.45A.). The agreement in this case was not so good, the difference is outside the experimental error of 6' of arc. This peak is consistently of higher Bragg angle than previously observed. It is suggested that since the previously observed results have been

obtained from photographs, the measured peak is in fact the mean of three peaks as shown on the trace. The peak observed by the other workers at 41° 40' (1.16 A.), is in fact a broad band extending over 1° 30', the mean centre of the band is at 41° 33' (1.16 A.). There is a similar band at 32° 6' to 34° 24'(a band width of 2° 18'), this band is also very weak. There is a weak but distinct peak at 27° 12'(1.69A.), this peak has not been recorded before. There are also five other distinct new peaks. -

11° 54'(3.57 A.) w.

14° 18'(3.12 A.) w.

19° 9' (2.35 A.) w.
19° 51'(2.27 A.) w. } twin peaks

20° 39'(2.18 A.)
21° 48'(2.07 A.) } w. band, possibly containing a fine structure of peaks.

Attempts to fit these observed new lines to the structure proposed by Astbury, of a monoclinic cell, were not successful. The calculated values of the Bragg angles for the selected reflections of the Astbury cell(see Ref. 8.) did not agree at all well with the observed angles. It was thought that this might have been due to the limited accuracy obtainable from these small charts so a thirty inches per hour chart trace was made.

POLYGLYCINE I X-RAY DIFFRACTION TRACE (20"per hour).

No.	Bragg θ°	d/n A.		strength.	Comment.
1	9° 15'	4.58		v.v.w.	
2	9 40	4.54		v.v.w.	
3	10 8	4.36	*	v.s.	Accepted value 4.4A.
4	10 44	4.12		v.w.p.	Possible Polyglycine II strongest peak.
5	11 36	3.81		w.sh.	
6	11 44	3.76		w.p.	
7	11 59	3.70	*	m.p.	
8	12 18	3.62		w.	
9	13 8	3.39		v.s.	Accepted value 12°55'(3.45A.).
10	14 0	3.19		w.	
11	14 18	3.14	*	w.	
12	14 34	3.06	*	w.	
13	14 52	3.00		w.	
14	15 28	2.89		w.sh.	
15	16 9	2.77		w.p.	
16	18 42	2.40		v.w.	
17	18 57	2.37		V.W. doublet	
18	19 4	2.35		v.w.	
19	19 10	2.34		v.w.	
20	19 35	2.29	*	w.	
21 [	19 45	2.28	*	w.]	2nd. order of 1)(2.29A.).
22 [	20 45	2.17	* band	v.w.	2nd. order of 3)(2.18A.).
23	21 11	2.13		v.w.	
24	21 25	2.11		v.w.	
25	21 28	2.09		v.w.	
26	21 55	2.06		v.w.sh.	
27	22 6	2.04		v.w.p.	
28	22 56	1.98		v.w.	
29	23 22	1.94		v.w.	
30	27 10	1.68	*	v.w.	2nd. order of 9)(1.69A.).

No.	Bragg θ°	d/n A.	strength.	Comment.
31	27° 25'	1.67	v.w.sh.	
32	27 41	1.66	v.w.p.	
33	27 58	1.64	v.w.	
34	29 2	1.59	v.w.	
35 [	29 13		v.w.]	
[	29 45	1.55	band.]	
36	30 15	1.53	v.w.	2nd. order of 12). 3rd. order of 1)(1.53A.).
37	31 52	1.45	v.w.	3rd. order of 3)(1.45A.).
38	32 21	1.44	v.w.	
39	38 38	1.23	v.w.	3rd. order of 7)(1.23A.).
40	39 47	1.20	v.w.	
41 [	40 24	1.19	v.w.] band	band contained peaks, mean of band at 41° 9'.
42	41 0	1.18	v.w.p.	This is lower than
43	41 20	1.17	v.w.p.	Bamford's peak at $41^{\circ}40'$.
44 [	41 38	1.16	v.w.] band	

Note.-

* denotes better defined peaks.

The results of the thirty inches per hour chart trace confirmed the differences observed above. The results are shown opposite; again a series of samples were used and the peaks recorded appeared on more than one chart. This trace also confirmed that the very strong peak appeared at 13° 8'(3.39 A.) as opposed tom the value obtained from the photographs of 12° 55'(3.45 A.), by Astbury and Bamford, and Meyer and Go[92]. In the light of this improved X-ray pattern it can be easily seen that the structure suggested by Astbury does not account for the observed peaks.

The unit cell is certainly not orthorhombic. It was therefore assumed that it was monoclinic as suggested by Astbury, and his proposed cell was modified so that a = 4.8 A. and angle β = 67° 21', this gave a peak at 10° 38' as the 100 reflection. Then either.-

θ_{010} = 11° 59' and b = 3.71 A.
θ_{001} = 13° 8' and c = 3.68 A.

or

θ_{010} = 13° 8' and b = 3.39 A.
θ_{001} = 11° 59' and c = 3.59 A.

either set of values for b and c produced some sort of agreement with the observed reflections for

reflections of the type h0l, but broke down completely for reflections of the type hkl. This seemed to suggest that the unit cell was triclinic.

Pauling and Corey(1953)[97] suggested that the structure was an antiparallel pleated sheet structure, with the ab-plane essentially as postulated by Astbury, with a fibre identity period of 6.96 A. to 7.0 A., the normal values for small peptides being about 7.23 A., If this is the correct structure the chains are not fully extended. Attempts to fit either of the limiting values for the fibre identity period into the X-ray diagram as a 020 reflection results in values for Θ_{020} which are much too low. A better figure for the fibre identity period is 6.79 A.. This then makes $\Theta_{020} = 13^{\circ}\ 8'$(v.s.), assuming a monoclinic cell. This did not give values for Θ, for reflections involving k, which agreed with those obtained from the chart.

CONCLUSIONS.

The structures which have been proposed for Polyglycine I do not agree with the results obtained here. There is no doubt that the material used here is that which is known as Polyglycine I. The unit cell is certainly not orthorhombic, and it most certainly is not the monoclinic cell proposed by

Astbury. A thorough investigation of possible values for the angle β, the monoclinic angle, for values close to 66°, the most promising value was found to be $\beta = 67° 21'$, but this value did not produce agreement with the observed values for reflections of the type hkl. Thus it is suggested that the true structure is triclinic.

This work cannot be further extended by powder photography, or by powder diffractometry. The best samples of the material which have been obtained are only poorly oriented and defined microcrystals. It is suggested that an attempt be made to obtain single crystals by recrystallisation of the hot water soluble Polyglycine I obtained from the polymerisation of glycine in phosphoric acid, see Exp. Sec. 8, p. 6.. This material is the first recorded instance of a hot water soluble low molecular weight polymer, assuming the Polyglycine I structure, and is worthwhile investigating in its own right.

POLYGLYCINE II.

This material was obtained in a much better crystalline form than the Polyglycine I. It was found that the repeated reprecipitation of the peptide, from solution in saturated aqueous calcium

chloride solution, by water; gave a well crystallised product. The material consisted of thin hexagonal platelets of microcrystalline size. It was not found possible to grow single small crystals by this method.

X-ray diffractometer traces of several specimens of the microcrystals were obtained; the peaks detailed overleaf are only those which appeared on more than one trace. There is no doubt that this material was pure Polyglycine II. A comparison was made with similar charts for both Polyglycine I and Pentaglycylglycine, run at chart speeds of three and thirty inches per hour. This showed that it was not contaminated by eithernof these peptides. The structure proposed by Crick and Rich(1955)[39] is reviewed in the Summary of Previous Work. This investigation was embarked upon in order to confirm this proposed structure.

The short three inches per hour charts showed initially quite good agreement with the proposed structure. Seven of the first eleven peaks of the proposed structure were accurately accounted for, within one percent in terms of d/n values. These peaks were (100),(101),(102),(003),(103),(110),(112), The peaks(111), and (201) were about 2% higher on

POLYGLYCINE II X-RAY POWDER DIFFRACTION PATTERN(30"/hr.).

No.	Bragg θ°	d/n A.	strength.	Comment.
1	9° 20'	4.75	v.w.	Crick and Rich peaks are given below.
2	9 41	4.57	v.w.	
3	10 36	4.17	*v.s.	(100)10°38'(4.15A.)←
4	11 10	3.96	v.v.w.	
5	11 30	3.87	w.	
6	11 36	3.82	*w.	(
7	11 47	3.77	w.	(101)11°42'(3.78A.)←
8	12 43	3.50	w.	
9	12 57	3.44	v.v.w.	
10	13 27	3.31	v.w.sh.	
11	13 36	3.28	w.p.	
12	13 47	3.23	m.sh.	
13	14 12	3.14	*m.p.	
14	14 24	3.10	*m.p.	(102)14°24'(3.10)←
15	14 38	3.05	*m.p.	(003)14°26'(3.09)← a sharp peak.
16	15 3	2.97	w.sh.	
17	17 30	2.56	v.w.	
18	17 44	2.53	v.w.	
19	18 0	2.50	*w.	(103)18° 5'(2.48A.).←
20	18 19	2.45	v.w.	
21	18 38	2.41	v.w. doublet	
22	18 42	2.40	w.	(110)18°43'(2.40A.)←
23	19 15	2.34	v.w.	(111)19°24'(2.32A.)‡
24	[19 31	2.30	v.w. band	
	[19 42	2.28	v.w.]	
25	20 46	2.17	v.w.	
26	21 3	2.14	*w. doublet	
27	21 10	2.13	*w.	(112)21°12'(2.13A.)←
28	[21 14	2.12	v.w. band	
	[21 24	2.11	v.w.]	
29	21 33	2.09	*w.	(200)21°50'(2.07A.).‡
30	[22 12	2.04	*v.w. band	

No.	Bragg θ°		d/nA.		strength.	Comment.
	22°	35'	2.01		*v.w.	(201)22°38'(2.02..)+
31	23	0	1.98		v.w.?	2nd. order of 4)?
32	23	10	1.96		v.w.?	
33	23	35	1.92		*w.	2nd. order of 5).
34	23	50	1.91		v.w.?	2nd. order of 6).
35	23	58	1.90		v.w.	(113)24° 1'(1.89A.) ω.
36	24	14	1.88		*w.	

* denotes better defined peaks.

? denotes ill defined peaks.

the chart than on the proposed values. The (113) peak was difficult to assign owing to three reflections occurring on the chart at about the right position.

The thirty inches per hour, long, traces were obtained in order to fix the postions of the diffractions peaks more accurately. They showed however, that the peaks which had been previously considered as single peaks, were in fact multiple peaks in many cases. The best defined examples of this fine structure of the peaks occurred at values of θ of about eleven and fourteen degrees, see Chart 13.. These multiple peaks are not due to statistical error, or variation of the X-ray tube output, since they appear in the same place on several traces. The hexagonal structure proposed by Crick and Rich requires that the $(10\bar{1}2)$ peak at $\theta = 14^{\circ}\ 24'$; should be stronger than the (0003) peak at $\theta = 14^{\circ}\ 26'$. On the chart trace shown a peak does occur at $14^{\circ}\ 24'$(m) but there is a stronger and better defined peak at $14^{\circ}\ 38'$. The proposed structure does not account for this peak at all. Neither does the structure account for the second peak at $11^{\circ}\ 30'$. There is also a significamt difference between the value of θ proposed for the (111) peak, $\theta = 19^{\circ}\ 24'$, and the peaks observed

at 19° 36'(w) and 19° 15'(v.w.). Again at the reflection for the (112) peak, θ = 21° 12', is the proposed value, but the chart showed that there are two possible peaks, θ = 21° 5' and 21° 10'. The proposed (200) reflection at 21° 50' has no corresponding peak on the chart, but a peak does occur at 21° 33'. The intensities of each of the proposed reflections have been calculated by Crick and Rich, these values were obtained from Crick. The calculations took into account the multiplicity of the reflections. The intensity of the(200) reflection should be lower than that of the neighbouring peaks, the chart shows that it is in fact about the same as that of the neighbouring peaks. If this peak is not that for the (200) reflection it cannot be accounted for by the proposed structure. A band occurs on the chart at 22° 12' to 22° 35', this should contain the proposed (201) reflection at θ = 22° 38'. This reflection should in fact be of the same intensity as its neighbours, whereas the observed intensity is much lower. There are twin peaks on the chart at 22°35' and 24°14'. The proposed (113) spacing has a θ value of 24° 1'. This would lie in the very weak band between these two peaks. There are no other

reflections in the structure to account for these observed peaks. The 23° 35' peak is the second order reflection of the peak at $\theta = 11^{\circ}$ 50', one of the other peaks not accounted for.

A critical comparison of the peaks observed on the chart and the peaks expected from the proposed structure showed that distinct differences existed. Over the range of θ values studied there are a total of eleven possible predicted reflections from the proposed structure. Seven of the reflections occur in the correct position, i.e. within one percent of the d/n value, these are marked thus ⊕. The remaining two proposed reflections do not have a corresponding reflection on the chart, and are marked thus ♯. There are in addition eleven other distinct peaks on the chart which cannot be accounted for by the proposed structure. The proposed structure is most certainly not that of the material of the specimen. The possibility that the structure was hexagonal but of slightly differing dimensions from those proposed, has been examined. Slight changes in the lengths of the axes of the hexagonal unit have not produced a better agreement between the predicted spacings and those observed. The electron micrographs of the material showed that the

crystals had at least an hexagonal cross section in the ab-plane. The possibility exists that the material is not homogeneous, but is in fact a mixture of two or more closely related crystalline forms of Polyglycine II; i.e. that Polyglycine II exists in two or more solid phases. Crick and Rich stated that their proposed structure was only the simplest member of a whole family of related structures. The examination of possible sizes of cell showed that if this were so the possible structures could not be hexagonal. If the material consisted of more than one phase, the phases must be of closely related structures, since they can crystallise together, and these crystallisations can be repeated without alteration of the X-ray pattern. A critical comparison with the observed diffraction trace for the pentaglycylglycine samples showed distinct similarities of basic structure, the electron micrographs of the two materials are very similar. This material does not however contain any low molecular weight pentaglycylglycine peptide since this would be easily soluble in the diluted calcium chloride solution resulting from the precipitation of the Polyglycine II. It was suggested by Crick in a private comunication, that the structure might deviate from that proposed if the

polymer chains ran both up and down, in either a random or a regular arrangement throughout the structure. The regular arrangement of the chains running up and down the structure of the cell would be expected to produce some extra spacings.

The remaining explanation is that the structure is a distorted hexagonal arrangement, and it belongs to the triclinic system. Attempts have been made to vary the angles between the a, b, and c axes of the crystals, but without any better agreements being obtained. This method is in any case a very haphazard approach, and is not very likely to be successful. The investigation of the structure by Bamford et alia[17] showed that the extended chain and the unrotated parallel polar sheet structures could be excluded on the grounds of the line intensities being wrong for the strongest reflections observed. They did however suggest a third alternative structure, the 2.2_7 helix of Donahue[46]. This structure gave line intensities in general agreement with those observed, but they did not agree in detail. In particular no 2.75 A. reflection was observed, corresponding to the residue repeat. The material investigated also showed no such reflection. They did state also that if the cell was triclinic this reflection would not be observed,

since there would be no corresponding lattice point. The predicted density for this structure was however 1.67 gm./ml. whereas the observed density is only 1.43 gm./ml..

CONCLUSIONS.

The structure is certainly not as simple as that proposed by Crick and Rich. The powder photographs and diffractometer traces obtained from this material are probably the best that can be obtained. The only way to obtain the true structure is by the method of single crystal X-ray analysis. This higher molecular weight Polyglycine II does not easily lend itself to the preparation of single crystals. It would probably be better to try first to prepare single crystals of the low molecular weight Polyglycine II obtained from the polymerisation of glycine in phosphoric acid, this can be obtained easily as a very good microcrystals. The pentaglycylglycine can also be obtained in a crystalline form very easily since it is hot water soluble. A single crystal investigation of this material, which shows a closely related diffraction pattern may help to elucidate the structure of Polyglycine II. The pentaglycylglycine structure may in fact be a limiting structure above which all the higher polymers take up the Polyglycine II form. On

the other hand the Polyglycine II form may itself be a mixture of two phases.

A MORE DETAILED EXAMINATION OF THE X-RAY PATTERNS OF THOSE COMPOUNDS SHOWING LOW ANGLE REFLECTIONS.

The compounds which showed low Bragg angle reflections in their X-ray patterns were all in the low molecular weight class. The average degree of polymerisation of each of the products was in fact about six. The materials were, the product of the polymerisation of benzoyl glycine and glycine, a benzoyl pentaglycylglycine, the product of the polymerisation of glycine in phosphoric acid, and pentaglycylglycine itself. Although the three diffraction traces showed some initial similarity, this proved to be only a superficial resemblence on closer inspection. The short three inches per hour traces were repeated using a chart speed of thirty inches per hour. This improved the accuracy with which the low angle reflections could be measured, but did not greatly improve the ease of measurement of the less intense peaks. No definite relationships between any of the three diffraction patterns could be drawn from the results, but the patterns are interesting since they are the first observed specimens to give such low angle reflections, it seemed almost as though these structures exhibit a superstructure.

BENZOYLPENTAGLYCYLGLYCINE.

This material was obtained by the polymerisation of glycine and benzoyl glycine, see Exp. Sec. 6., The X-ray diffraction trace showed a peak at 1° 34', 28.0 A.(m), this corresponds to a peak at 1° 39' on the trace of the product from the polymerisation of glycine in phosphoric acid. The second order of this peak appeared at 3° 9', 14.1 A., the third order at 4° 45', 9.3 A., the sixth order at 9° 31', 4.67 A., and the eighth order at 12° 49', 3.47 A.. None of these higher order peaks were stronger than very weak. The strong peaks on the trace occurred at 10° 34', 4.18 A.(v.s.), this corresponds to the 10° 38', 4.15 A.(v.s.), peak for Polyglycine II, and pentaglycylglycine. The remainder of the peaks on this trace are weak or very weak. The peaks are shown opposite.

THE PRODUCT FROM THE POLYMERISATION OF GLYCINE IN PHOSPHORIC ACID.

This material was obtained from the product of the polymerisation of 40 gm. of glycine in 5 ml. of concentrated phosphoric acid. This material was extracted by the second wash with hot water, of the reaction mixture, see Sec. 8.,p. 4.. This material gave an X-ray diffraction pattern showing several

THE X-RAY DIFFRACTOMETER TRACE OF BENZOYLPENTAGLYCYLGLYCINE.

No.	Bragg θ°		dθnA.	strength.	Comment.
1	1°	34'	28.0	m.	1° 39'peak in G/H_3PO_4.
2	3	4	14.5	W doublet	
3	3	9	14.1	w.	2nd. order of 1).
4	4	43	9.3	v.v.w.	3rd. order of 1).
5	5	0	8.85	v.v.w.	5° 5'peak in G/H_3PO_4.
6	5	15	8.45	v.v.w.	5° 9' ,, in G/H_3PO_4.
7	6	40	6.65	v.v.w.?	
8	8	39	5.12	v.v.w.	6th. order of 1).
9	9	31	4.67	v.v.w.	9°34'peak in G/H_3PO_4.
10	10	34	4.18	v.s.	10°38'(4.15A.)peak in P.G.II and G_6.
11	11	10	3.96	w.	11°10'(w) peak in G_6.
12	11	22	3.89	w.	
13	11	36	3.81	w.sh.	11°36'(w)peak in G_6 & G/H_3PO_4
14	11	45	3.76	w.sh.	
15	12	0	3.71	w.	11°56'(w)peak in G_6.
16	12	12	3.64	w.sh.	
17	12	27	3.57	v.w.	12°29'(w)peak in G_6.
18	12	49	3.47	v.w.	8th. order of 1).
19	13	6	3.40	v.w.sh.	12°42'peak in G/H_3PO_4.
20	13	17	3.35	v.w.sh.	
21	13	46	3.24	v.w.sh.	13°46'(w)peak in G_6.
22	13	56	3.20	v.w.	
23	14	16	3.13	v.w.	
24	14	30	3.08	v.w.	14°26'(m)peak in G_6.
25	14	34	3.06	v.w. band	
	15	3	2.97	v.w.	
26	17	7	2.64	v.w.	17°10'(v.w.)peak in G/H_3PO_4.
27	17	20	2.59	v.w. band	17°26'(v.w.)peak in G/H_3PO_4.
	18	4	2.48	v.w.	18° 0'(v.w.)peak in G/H_3PO_4.

THE X-RAY DIFFRACTION PATTERN TRACE OF THE PRODUCT OF THE POLYMERISATION OF GLYCINE AND PHOSPHONIC ACID.

No.	Bragg θ°	d/n A.	strength.	Comment.
1	1° 39'	26.75	v.s.	
2	3 26	13.60	m.	2nd. order of 1).
3	5 5	8.68	w. doublet	
4	5 9	8.57	w.	
5	5 18	8.34	v.w. doublet	
6	5 26	8.13	v.w.	
7	9 34	4.62	v.v.w.	
8	10 47	4.10	v.v.w.	Polyglycine II & G_6(v.s.).
9	11 36	3.81	v.v.w.	G_6 peak(w.)
10	12 52	3.46	v.v.w.	
11	13 42	3.22	v.s.	8th. order of 1). weak peak in G_6(v.w.).
12	14 5	3.17	m.	G_6 peak (14° 0')(w.).
13	14 24	3.10	m.	G_6 peak (14°26')(m.).
14	14 35	3.06	m.	
15	15 18	2.92	v.s.	9th. order of 1). 16°56'peak in G_6(v.w.).
16	16 53	2.66	v.w. doublet	
17	16 57	2.64	v.w.	16°56'peak in G_6(v.w.).
18	17 10	2.61	v.w.	
19	17 26	2.57	v.w.	
20	17 36	2.55	v.w.	
21	18 0	2.50	v.w.	
22	21 30	2.09	v.w. doublet	
	22 11	2.04	v.w.	
23	22 12	2.04	v.w. doublet	
	22 42	2.00	v.w.	
24	24 40	1.85	v.w. band	
	25 57	1.76	v.w.	

strong lines. The first peak appeared at a Bragg angle of 1° 39', 26.7 A.(v.s.), this corresponds to the peak in benzoylpentaglycylglycine. The second peak was at 3° 26', 13.6 A.(m), this was the second order peak for the first reflection. The next strong peak appeared at 13° 42', 3.25 A.(v.s.), this could have been the eighth order peak of the first peak, the error being only three percent, in the value for d/n.. There are three possible peaks at 14° 5', 3.17 A.(m), 14° 24', 3.10 A.(m), 14° 35', 3.06 A.(m). A 14° 24' peak, 3.10 A.(m), appeared in the trace for Polyglycine II, and for pentaglycylglycine. A strong peak also appeared at 15° 18', 2.92 A.(v.s.), this corresponds to the ninth order of the first peak. Apart from the one peak mentioned above, the trace showed no resmblence to that of either Polyglycine II or Pentaglycylglycine.

PENTAGLYCYLGLYCINE.

This material was the same as that used for the work of Exp. Sec. 15.. The diffraction trace although different from that of Polyglycine II, showed a considerable number of common peaks. The pattern also showed many high order reflections of the low angle reflections. The first reflection occurred at 1° 53', 25.8 A.(m.sh.), the second at

THE X-RAY DIFFRACTOMETER TRACE OF PENTAGLYCYLGLYCINE.

No.	Bragg θ°	d/n. A.	strength.	Comment.
1	1° 53'	25.8	m.sh.	shoulders to the zero, undeviated beam peak.
2	2 7	20.9	m.sh.	
3	3 45	11.7	v.v.w.	
4	4 14	10.45	v.w.	2nd. order of 2).
5	6 24	6.90	v.v.w.	3rd. order of 2).
6	6 48	6.50	v.w.	4th. order of 1).
7	9 40	4.57	v.w.	
8	10 6	4.42	v.w.	
91	10 39	4.15	* v.s.	Polyglycine II(10°38'). 5th. order of 2).
10	11 10	3.94	* w.	3rd. order of 3).
11	11 32	3.83	* w.	Polyglycine II(11°42').
12	11 56	3.71	* w.	
13	12 29	3.67	v.w.	7th. order of 1).
14	12 53	band	v.w.	
15	12 57	3.44	v.w.p.	6th. order of 2).
16	13 6	3.40	v.w.p.	
17	13 10	band	v.w.	
18	13 46	3.24	* w.	8th. order of 1).
19	14 0	3.19	w.	
20	14 26	3.09	* m.	Polyglycine II(14°24').
21	15 9	2.95	* w.	7th. order of 2). 4th. order of 3).
22	16 2	2.79	v.w.	
23	16 56	2.65	* v.w.	8th. order of 2).
24	17 43	2.53	* w.	
25	18 41	2.40	* w.	Polyglycine II(18°43').
26	19 28	2.31	w.	Polyglycine II(19°24').
27	20 14	2.23	v.w.band.	
28	20 45	2.17	v.v.w.	
29	21 43	2.08	v.w.band	Polyglycine II(21°50').
30	22 26	2.02	v.v.w.	Polyglycine II(22°38').
31	23 7	1.96	*v.v.w.	6th. order of 3).
32	23 37	1.92	v.w.band.	
33	23 57	1.90	*v.w.	Polyglycine II(24° 1').

2° 7', 20.9 A.(m.sh.). The first reflection occurred as the fourth, seventh, and eighth orders. The second reflection occurred as the second, third, fifth, and the sixth to the tenth orders. The fourth order was absent. The very strong peak at 10° 39', 4.15 A., corresponded to the fifth order of the second peak. This peak also corresponded to the very strong peak in the Polyglycine II trace. A medium strength peak at 14° 26', 3.09 A., in the trace corresponded to the 14° 26', 3.09 A., peak in Polyglycine II, both being of similar intensities. The comparison of the pattern with that for Polyglycine II, as shown in the table of reflections, shown opposite. Some attempts have been made to interpret this diffraction pattern in terms of a hexagonal structure, without success. The first and second peaks are certainly main spacings since they are repeated to very high orders. It is however very difficult to assign a third spacing for the unit cell. The 11.7 A. reflection, does not show the same certainty of high order repetition. This trace certainly shows that the structure of Pentaglycylglycine is no simpler than that of Polyglycine II, The ease with which Pentaglycylglycine can be crystallised seems to suggest that this material would be ideal for a

single crystal X-ray investigation. The structure would be extremely interesting in view of the low angle spacings, and in view of the similarity of the structure with that of Polyglycine II. Polyglycine II certainly does not show any low angle reflections, all the samples which have been prepared by the author have been checked to ensure that this is so.

THE DISCUSSION OF THE RESULTS.

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THE SEARCH FOR NEW FORMS OF POLYGLYCINE.-

The search for new forms of Polyglycine has not yielded any new crystalline modifications of the polymer, although this does not preclude the existance of new forms. The methods of preparation have all yielded products which were non-homogeneous as regards their chain length. When it has been possible to carry the reaction to completion, the end product of most of the polymer preparations has been Polyglycine I. The Polyglycine I does however, contain a small quantity of Polyglycine II; it has never been possible to remove the X-ray diffraction lines of Polyglycine II, completely from the X-ray photograph of any Polyglycine I specimen. This has been attempted by prolonging the reactions and by heating Polyglycine I in various solvents. Both of these methods have been shown to convert Polyglycine II, of degree of polymerisation of 10, into Polyglycine I. The intensity of the Polyglycine II lines shown by the Polyglycine I, have been reduced by this means, but never completely eliminated. The results from heating Polyglycine II. in water, has shown that conversion of the Polyglycine II into

Polyglycine I has been quite rapid. It seems therefore that the Polyglycine I contains a form of Polyglycine II, which is not easily capable of conversion to Polyglycine I, or its rate of conversion is much slower than in the case of normal Polyglycine II(reference .- Exp. Sec. 9). It is suggested that the Polyglycine II obtained on cooling the hot filtered aqueous extract of Polyglycine I(reference .- Exp. Sec. 9.) may be this more stable form of Polyglycine II. This material like that obtained from the hot aqueous extract from the polymerisation of glycine in phosphoric acid(reference.- Exp. Sec. 8.), is distinctly different from normal Polyglycine II. It is soluble in hot water, whereas normal Polyglycine II is only soluble in strong aqueous salt solutions. It does however show the same hexagonal structure, with growth steps, under the electron microscope. The X-ray diffraction photographs are also identical. It is a peptide of lower molecular weight than normal Polyglycine II. The degree of polymerisation must be in the region of 6 to 10 for the material to be water soluble. It is possible that the lower peptides of glycine although having their own distinct structure when crystallised alone, crystallise out from the

reaction mixture as mixed crystals, having the structure of Polyglycine II. The structure of the lower molecular weight peptides shows the strong 4.15 A. spacing of Polyglycine II; for example glycine hexapeptide(reference.- Exp. Sec. 13) and the benzoylated peptide(reference.- Exp. Sec. 6.) shown in this thesis, and the penta-, hexa-, and hepta-peptides of glycine investigated by Meyer and Go[92]. It may therefore be that the stable structure of these lower peptides is the Polyglycine II structure in preference to the Polyglycine I structure. Although low molecular weight Polyglycine I has also been obtained from the product of the polymerisation of glycine in phosphoric acid. More work would have to be done on this point to be sure that this was so. A few tentative experiments were carried out on the crystallisation of low molecular weight peptide mixtures, but the range of peptides available was too small to reach any conclusions. The hot water soluble form of Polyglycine II was extracted from other samples of Polyglycine I as well as those obtained as described above, it was also obtained from the residue from the preparation of diketopiperazine. The best yield is obtained from the reaction product of glycine

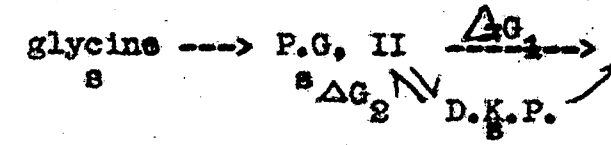
and phosphoric acid. No other sample of water soluble Polyglycine I, than that discussed above, was obtained.

The examination of the reaction in which glycine is directly polymerised to Polyglycine has also yielded information about the reaction mechanism. It has been shown, in several of the methods used, that the Polyglycine II form was obtained as the initial product. In the study of the polymerisation of glycine in hydrochloric acid (reference.- Exp. Sec. 2.) it has been observed that the Polyglycine II was formed in the liquid phase. It was also confirmed that solid glycine must be present in the reaction mixture for polymer to be formed. The Polyglycine II formed initially was then converted into Polyglycine I, in the reaction mixture, by more prolonged heating. The transition of Polyglycine II into Polyglycine I does not occur on heating the dry polymer. The presence of a liquid phase is essentialk in order to bring about the transition. This transition will be more fully discussed later. The effect of dry heat on the Polyglycines has been carefully studied. Leggy and Sims[90] found that heating Polyglycine I reduced its absorption of the dye Orange II, by about 33%. The

effect of dry heat has been shown to produce no change in the X-ray structure of either form of Polyglycine. It is possible that the transition of Polyglycine II into Polyglycine I does occur in the solid phase, and that the water or liquid phase, merely acts as a catalyst. There is some support for this idea since the transition occurs at temperatures down to 72° C., whereas the solubility of Polyglycine II would be very low, in aqueous media. However in contradiction of this, if Polyglycine II is heated with 20% aqueous calcium chloride solution at 72°C. it is found that some of the polymer is converted into Polyglycine I. no conversion occurred using 10% calcium chloride solution. The polymer was known to be just soluble in the 20% solution at this temperature, and insoluble in the 10% solution.

The products of the polymerisation of glycine in hydrochloric acid have also been shown to contain diketopiperazine. This was suspected from some of the X-ray photographs of polymerisation products, and was confirmed by chromatographic examination of the reaction products. The diketopiperazine in the reaction mixture could have been produced at two possible stages in the reaction . Firstly, in the initial formation of Polyglycine II from glycine. This does not agree with the reaction

mechanism proposed by Meggy[88], whereby the glycine was initially polymerised to glycylglycine. He also showed that the addition of glycylglycine to the reaction mixture, resulted in an increase of the reaction rate, and gave this as the main reason for proposing this initial step. Secondly it is postulated that, the relationship between diketopiperazine and Polyglycine is a reverisble ring-chain equilibrium, which is disturbed by the hydrolysis of diketopiperazine to glycine. No diketopiperazine is present in the solid phase of the reaction mixture. It is not possible to say which of the two Polyglycines is in equilibrium with diketopiperazine. From the work of Meggy[88], Polyglycine, it was not then known that two forms were present, was thought to be in equilibrium with a saturated solution of diketopiperazine. Thus perhaps the relationship between the Polyglycines and diketopiperazine can be represented by .-



Now $\Delta G_1 = \Delta G_2 + \Delta G_3$

and from the work of Meggy[88], ΔG_3 is about -500 cal. i.e. very small. Also from the relative solubilities of Polyglycine I and II in saturated aqueous calcium chloride solution, which gave a value of about 1 : 2

the value of ΔG_1 is approximately -RT.ln 2.

Thus $\Delta G_1 = -(2 \times 420 \times 2.303 \times 0.30)$

$\Delta G_1 = -580$ cal.

Thus it is possible that the value for ΔG_2 may be very small and may approach zero. Hence the equilibrium solution for the reaction, could be saturated with diketopiperazine, the solubility of diketopiperazine is 10% at 100°C. in water. On cooling the reaction mixture this diketopiperazine would crystallise out, its solubility in water is only 1.4 % at 20°C. This could account for the presence of very weak lines for diketopiperazine in the X-ray diagrams of some of the solid polymers. The polymerisation of diketopiperazine in water (reference.- Exp. Sec. 7) shows that this polymerisation follows the same general mechanism as that shown above. But the X-ray diffractometer traces of the reaction products show that in all cases only some of the strong diketopiperazine lines are present, and only certain line intensities have been reduced. This seems to indicate that the Polyglycine II formed initially is formed in the solid state at the expense of diketopiperazine, solid. The Polyglycine II seems to be formed by rearrangement of the diketopiperazine lattice along one, at least, of its

crystallographic axes. If this reaction occurred in solution it would be expected that some diketopiperazine would remain in its original crystalline state, this is not observed on the traces. This partial weakening of lines in the X-ray pattern of diketopiperazine has also been observed in products obtained from the polymerisation of glycine in phosphoric acid. This reaction also occurs by the typical mechanism for direct polymerisation of glycine. This discovery favours the first proposal, and may indicate that glycylglycine cyclises to diketopipŝerazine very readily, and that the diketopiperazine ring is only opened under strongly acid conditions. Thus diketopiperazine is the preferred intermediate, to glycylglycine, in the polymerisation mechanism. The intensity of the diketopiperazine lines continues to diminish as the Polyglycine II formed initially is converted into Polyglycine I, thus it is not a significant intermediate in the interconversion reaction. The molar yield curve for the polymerisation of glycine in hydrochloric acid is typical for a reaction which is autocatalytic, it also shows an initial induction period. The polymerisation of glycine in glycerol and glycine in phenol follows exactly

the same pattern as the glycine and hydrochloric acid polymerisation, except that the rate of polymer formation is much reducdd. It seems therefore that the liquid phase of the reaction mixture acts as a combined solvent and catalyst for the polymerisation. The conversion of the initially formed Polyglycine II into Polyglycine I was more complete in the case of polymerisation of glycine in hydrochloric acid , than in the case of the other polymerisations.

The polymerisation of glycine in glycerol (reference.- Exp. Sec. 3.), showed that the proportions of Polyglycine I and II in the product depended on the volume of the liquid phase. Where a large volume of glycerol was added the reaction rate was increased but a high proportion of Polyglycine II was obtained in the product, e.g. Exp. Sec. 3 p. 10(iii). Whmere the proportions were as in reaction (i)p.9., 5 gm.per 2 ml., the reaction rate was decreased, but a higher proportion of Polyglycine I was obtained. It appears that the formation of Polyglycine II is dependent on having a large volume of liquid phase, in the presence of solid glycine, this would seem to indicate that the Polyglycine II is formed in solution in the liquid phase. It also shows that the conversion of

Polyglycine II into Polyglycine I only occurs when its concentration in the mixture, or in the liquid phase, reaches a certain limiting value. Polymerisation of glycine in glycerol diluted with water, gave a lower yield of polymer, and the product in these cases was always Polyglycine II only. Using 25% glycerol in water, mole ratios glycine: glycerol: water, 0.748 : 0.019 : 0.23, the yield of polymer was less than 1%, and an X-ray photograph of the wet reaction mixture showed lines for glycine and diketopiperazine as well as the weak Polyglycine II lines. Thus as the activity of water in the system increased the reaction leading to polymer formation was repressed. Diketopiperazine was formed instead of Polyglycine II. This is in agreement with the usual method of preparation of diketopiperazine from glycine in excess glycol. This reaction was also found to produce small quantities of Polyglycine II which was soluble in hot water and so of low molecular weight. The brown fluorescent material produced in these polymerisations will be discussed separately. It is of interest that glycine can also be polymerised to diketopiperazine by heating 700 gm. of glycine in 3.5 l. of glycerol. The hot 160°C. solution contains no solid diketopiperazine, this

only crystallises out on cooling. If however the high solvent ratio is reduced solid Polyglycine I and II are obtained.

The polymerisation of glycine in the phenol rich phase of the phenol water system(reference.- Exp. Sec. 4.), followed the same course as the other reactions discussed above. It was found that a very well crystalline sample of Polyglycine I, could be obtained by this method. The slow rate of formation of the polymer aided the growth of Polyglycine I crystallites. All the samples obtained by other polymerisation reactions were shapeless masses of crystallites; the samples obtained by this method did at least show rectangular cleavage. Since no method for the precipitation of high molecular weight Polyglycine I, as good crystals has been found , this method could be made to produce the best product as regards crystalline quality. The more usual product from this reaction was a mixture of Polyglycine I, together with some Polyglycine II. The proportion of Polyglycine II obtained in this reaction, again depended on the volume of the liquid phase. Tarry products were produced in addition to polymer, and the tubes showed much pressure on opening, carbon dioxide gas was dissolved under pressure in the solution. The tarry products were soluble in ethanol.

The solution from the reaction was allowed to stand for two weeks, and the solid which was deposited was was examined by X-ray diffraction. The solid contained glycine and diketopiperazine . This again agreed with the results obtained from the other polymerisation methods. Polymerisations did not occur in anhydrous phenol, or in the water rich phase of the phenol water system. This may have been because the reaction rate was much slower in these media. At equilibrium at room temperature, the activity of water is the same in both phases of the phenol water system. At 140°C. the system approaches more ideal behaviour, the critical solution temperature is at 67°C.. The activity of water is much lower in the phenol rich phase at 140°C,, than in the water rich phase. Thus the reaction may not take place in the water rich phase.

The formation of Polyglycine by the polymerisation of glycine in the various media, may safely be assumed to conform to one pattern of reactions. The rate of reaction appears to depend on the activity of water in the medium at the reaction temperature, and upon the other constituents of the reaction medium. The large differences in reaction rates observed with different media suggests that the media acts as a catalysts. This is most certainly true in the case

of polymerisation of glycine in hydrochloric acid. A second factor should also be considered, namely the solvent action of the medium used. The reaction occurs much faster in hydrochloric acid, the overall yields of polymer are higher and the proportion of Polyglycine I is greater, than in the case of other media. Acids and salt solutions are known to be good solvents for the Polyglycines at ordinary temperatures, ~~xxxxxxx~~. The other media studied, are not solvents for the Polyglycines at ordinary temperatures at least, although they may act as solvents at elevated temperatures. The formation of Polyglycine I from Polyglycine II has been shown to require the presence of an aqueous phase. Thus it is probable that this conversion is speeded up in the presence of a better solvent than water. Water has been shown by Meggy[89] to act as a solvent at 140°C. If this reaction rate is increased then from Le Chateliers Principle, the rate of formation of Polyglycine II may also increase. The increase of the concentration of acid above the optimum proportions was shown by Meggy to lead to lower yields of polymer. He showed that excess acid brought all the glycine into solution at 140°C.. Glycine must be present as a solid phase for Polyglycine to be formed, because ΔG is only slightly negative for this reaction, and it becomes

positive for solutions of glycine. Thus the addition of acid beyond a certain limit leads to a decrease in the rate of formation of Polyglycine II. The activity of water in the system then controls the rate of formation of Polyglycine II. from glycine. The presence of water in the system seems to be essential for polymerisation to occur, since no polymer was obtained from solutions of mixtures of glycine and anhydrous phenol. The heating of glycine alone(refernce.- Exp. Sec. 5.) also showed that an aqueous phase was necessary for polymerisation to occur. Glycerol is capable of replacing water in the reaction, since it is hydroxylic and a good solvent for the reaction. Phenol although hydroxylic is not such a good solvent as water or glycerol, for either the Polyglycines or glycine. The observation that diketopiperazine appears as an intermediate in the reaction further complicates the interpretation of the mechanism for the reaction.

The polymerisation of glycine by heating alone also gave Polyglycine I only. The reaction was slow, 2 weeks at 170°C.. This reaction is very tempermental and seemed to depend on the glycine being slightly damp with water. Since water is formed during the

course of the polymerisation, the reaction is probably autocatalytic. The polymer from this reaction showed a considerable "leafing effect", again it had been allowed to grow slowly.

The polymerisation of glycine in phosphoric acid at atmospheric pressure has been shown to produce both Polyglycine I and II according to the length of time for which the reaction was allowed to proceed. The novelty of this method was in the range of polymers of different degrees of polymerisation, which can be obtained. In particular forms of both Polyglycine I and Polyglycine II of low molecular weight were obtained, these samples were of very good crystalline quality. The polymerisation could also be carried out in sealed tubes as well, the products were again of a wide range of degrees of polymerisation. In particular it showed that it might be possible to obtain polymers of higher molecular weights than the Polyglycines obtained here. The reaction mechanism appeared to be very similar to that proposed for the other polymerisation methods. The product was also shown to contain the partially converted diketopiperazine which had been observed in the products from some of the other polymerisations. This polymerisation reaction can

be controlled, so that Polyglycine II only is obtained. This method also yields the best yield of low molecular weight Polyglycines. It is the only polymerisation which has produced a low molecular weight form of Polyglycine I. It has also produced another new low molecular weight polymer showing low Bragg angle spacings, and thus a large unit cell. The only other method which has been recorded for the preparation of Polyglycines by polymerisation at atmospheric pressure. This is the method of Heggy[89], using glycine and hydrochloric acid, which he states to be of low and variable yield. This new method is much superior as regards the yield obtained.

The direct polymerisation of glycine seemed to follow the same general mechanism in all the cases examined. The presence of the diketopiperazine showing some X-ray lines of reduced intensity, in some of the products, shows that it is an intermediate in the formation of Polyglycine II. It also suggests that the Polyglycine II is not formed in solution, but is formed by direct polymerisation of the diketopiperazine in the solid state. If this is so the reaction may be catalysed by acid catalysts, since such a catalyst would be expected to cause ring

opening of the diketopiperazine. It has been shown that increasing the volume of the liquid phase has stopped the reaction at the diketopiperazine stage, no further polymerisation being possible. The investigation of this reaction is far from complete, and it is not possible to reach any firm conclusions at this stage.

THE BROWN FLUORESCENT MATERIAL PRODUCED DURING THE POLYMERISATION OF GLYCINE IN GLYCEROL.

It was found that all the polymerisations of glycine in glycerol(reference.- Exp. Sec. 3.), produced a brown fluorescent material in addition to the polymer. This material was soluble in the glycerol, and in the water used to extract the material from the Carius tube. This brown colour could be partially extracted from the solution, by the addition of acetone. The acetone extract was evaporated down leaving a thick dark brown oil. The remaining brown colour in the aqueous solution, could then be removed by the addition of ether. The extraction of them solution initially with ether had not been effective. Thus the acetone must have reacted with some compound in the solution, as well as acting as an extracting medium. Acetone will

react with compounds containing active methylene groups, or amino groups. The evaporation of the ethereal extract left a brown green fluorescent oil. It was not possible to induce either of these oils to crystallise. This observation agreed with the suggestion of Maillard, that the brown material consisted of two compounds. These oils can be easily separated and would provide an interesting topic for research.

THE RED-BROWN COLOUR PRODUCED DURING THE PREPARATION OF DIKETOPIPERAZINE FROM GLYCINE AND GLYCOL.

During the preparation of diketopiperazine by heating glycine in glycol(reference.- Exp. Sec. 3.), the glycol gradually became a dark red-brown colour. This colour could be extracted by the addition of 3 to four times the volume of acetone. In contrast to the material discussed above, this material separated on the walls of the vessel as a dark brown crystalline precipitate. A little colour still remained in the glycol layer. The X-ray diffraction photograph showed that this material was neither glycine not diketopiperazine, nor was it a mixture of these compounds. The line pattern could not be

related to any of the peptides or peptide ester patterns which have been obtained. This material is easily separated and may help to elucidate the mechhanism of the dimerisation reaction. It may also help to explain the low yields of diketopiperazine obtained in this reaction. It would be far less troublesome to deal with than the oils obtained above, and would be even more rewarding as a topic for investigation.

THE TRANSITION OF POLYGLYCINE II TO POLYGLYCINE I.

It has been shown that this transition of Polyglycine II into Polyglycine I occurs in the polymerisation reactions involving the direct polymerisation of glycine. The transition occurred at 140°C. in a variety of solvents.

It was claimed by Leggy and Sikowski[91], that a solution of Polyglycine I in saturated aqueous calcium chloride solution, when precipitated by water at temperatures above 60°C., gave a product containing some Polyglycine I. Below 60°C. Polyglycine II only was precipitated from the polymer solution. They did however state that Polyglycine II dissolved in calcium chloride at 100°C, and precipitated at 100°C. gave Polyglycine II only, no transition had occurred.

This work has been extensively checked, and no trace of such a transition has been found. The precipitation has been studied at temperatures between 60°C. and 120°C.. At all these temperatures Polyglycine II only was obtained. The concentration of the Polyglycine solution, the extent of dilution with water, and the pH of the polymer solution were varied, still no transition was observed. The claim that there is a transition temperature at 60°C. is therefore erroneous.

Precipitation of the Polyglycine I solution in saturated aqueous calcium chloride solution, was carried out using a number of other precipitants. Methyl and ethyl alcohols and glycerol produced products which contained some Polyglycine I as well as Polyglycine II . Variation of the precipiation conditions had little effect on the proportions of the two polymers obtained. Precipitation by ethyl alcohol, produced a gum like mass, which was extremely difficult to separate. The use of dimethylformamide and 0.88-ammonia as precipitants gave Polyglycine II only as products.

Aqueous 70% zinc chloride solution was also used as a solvent for Polyglycine I. Precipitation of this solution by water under various conditions,

and at temperatures between 20°C. and 100°C. produced only Polyglycine II. Precipitation of this polymer solution by ethyl alcohol produced a product which showed ill-defined, diffuse, X-ray lines characteristic of Polyglycine I only. The diffuse character of the peaks on the diffractometer trace suggested that the material was not very well crystalline. The poor crystalline quality of the product suggested that the precipitation had occurred rapidly, and that the rate of nucleation had greatly exceeded the rate of crystal growth. The precipitant was added rapidly, but the time taken for a precipitate to appear was usually about 5 to 10 minutes. An excess of ethyl alcohol, 60 ml., per 5 ml. of polymer solution, was required for the precipitation of Polyglycine I. It was noticed that the mixture became very hot, i.e. a high heat of mixing, just after mixing the solutions.

It is not possible to say what form the Polyglycine is present as in the polymer solution. Both the Polyglycine I and II are only defined as crystalline solids. Polyglycine II is the form in which the Polyglycine usually crystallises out, first of all, from the solution. It has been shown that this is the metastable form, but the rate of conversion to the stable Polyglycine I form, is extremely slow at room temperature. Thus there are several possible

explanations for the precipitation of Polyglycine I directly from the solution. The high thermal energy released as the heat of mixing may cause sufficiently intense local heating in the solution to either accelerate the conversion of initially precipitated Polyglycine II, or even result in the direct precipitation of the stable Polyglycine I. Alternatively the specific effect of the zinc ions and the ethanol have to be considered. The zinc ions (Zn^{++}) may catalyse the transformation of Polyglycine II to Polyglycine I. Or again it may cause the direct precipitation of Polyglycine I. Zinc chloride solution is distinctly acidic, and also zinc ions show an enhanced ability to coordinate with nitrogen , e.g. zinc chloride and pyridine salts. Similar arguments can be made out for catalysis by ethanol. It cannot be argued that the Polyglycine I structure was partially retained in solution, since a solution of Polyglycine II on precipitation by alcohol, also gave Polyglycine I as the product. All the other precipitating agents have produced products of Polyglycine II only from solutions of Polyglycine II, except methanol, which produced a mixture of both forms of the Polyglycine. It is not possible at this stage to determine the form in which the Polyglycine is present in solution, or to state the exact

mechanism for the precipitation reaction. The effect cannot be merely due to the ethyl alcohol precipitant since with calcium chloride solutions of the polymer, only mixtures of Polyglycine I and II were obtained.

Cuprammonium hydroxide solution was also found to be a solvent for the Polyglycines. It was found that some of the Polyglycine could be precipitated from the solution by ethyl alcohol as a cuprammonium complex. The products obtained from solutions of Polyglycine I and Polyglycine II, differed only in the intensity of one line in their X-ray photographs. These products have been shown to be true complex compounds. They are of interest since this is one of the few recorded cases of compound formation by the Polyglycines. The complexes were decomposed by the action of dilute sulphuric acid. The products showed, that in the case of Polyglycine I, considerable change had occurred to Polyglycine II, the Polyglycine II complex gave Polyglycine II only. This showed that the Polyglycine ~~II~~ I structure had at least been partially retained in the cuprammonium solution.

Heating the Polyglycines in the open air oven resulted in no interconversion. There was however, a 3% loss in weight in both cases. This was at first thought to be due to the moisture regain of the

polymer. On repeating the experiments, but this time heating in a sealed tube, similar losses in weight were noted. The polymer did not appear to be at all damp, nor was there any trace of moisture on the walls of the tube. The tubes in both cases showed some pressure on opening. The X-ray photographs of the products showed that no interconversion had occurred, and the sharpness of the lines showed that there had been no appreciable change in crystalline quality, both Polyglycines had however become a pale buff colour. It was assumed that some of the lower peptides which have been shown to be present had decomposed.

Polyglycine I heated in a sealed tube with water, showed no change in crystal structure even on prolonged heating, although the Polyglycine II lines which were always present in the material, did decrease slightly, in intensity, on prolonged heating. It was not possible to eliminate these lines completely, by heating with water. Polyglycine II was converted into Polyglycine I, at least partially, at all temperatures between 72°C. and 150°C., on heating with water in a sealed tube. The rate of conversion at 72°C. was very slow, raising the temperature to 120 °C. produced a considerable

increase in the rate of conversion. Raising the temperature a further 30°C. produced a sixfold decrease in the time required to produce the same yield of Polyglycine I. Thus if the Polyglycine II which was present in the Polyglycine I were "normal" Polyglycine II, it would be expected that it would be converted completely to Polyglycine I on prolonged heating. This suggests that the Polyglycine II, in the Polyglycine I, is the low molecular weight material which can be separated from it by solution in hot water. This low molecular weight material may be stable in the Polyglycine II structure, and not affected by heating.

The comparitively large temperature range over which the conversion of Polyglycine II into Polyglycine I occurs, suggests that there is no true transition temperature for this conversion. The Polyglycine II is a metastable form. This is supported by the fact that the conversion can only be carried out in one direction, from Polyglycine II to Polyglycine I. The only method available for the conversion of Polyglycine I into Polyglycine II is by the precipitation of a solution of the polymer. The fact that Polyglycine II is present as an intermediate in the formation of Polyglycine I also

supports the idea that it is the metastable form. The solubility of Polyglycine II in all the solvents investigated was always much greater than that for Polyglycine I. This again would be the case if Polyglycine II were metastable. This is shown in the case of solutions of the polymer in calcium chloride solution. The solubility of Polyglycine II is about twice that of Polyglycine I.

The aqueous solutions obtained from heating the polymers in water were also examined. It was found that in the case of Polyglycine I, some of the polymer had been decomposed to diketopiperazine. In the case of Polyglycine II, the aqueous solution, contained only glycine. Thus it is possible that the two Polyglycines are decomposed by different mechanisms.

The Polyglycines were also heated in a saturated, 20%, and 10% solution of calcium chloride in water. The saturated solution completely hydrolysed the polymers even at 72°C. The resulting solution was unfortunately not examined. Heating the polymers in the 20% solution resulted in some hydrolysis. Polyglycine I remained unchanged, but some polymer was lost. Polyglycine II was partially converted into Polyglycine I. It was known that at 100°C. the Polyglycine II was only just soluble in 20% calcium

chloride solution. Thus the rate of interconversion would be expected to be slow if it occurred in the solution. Heating Polyglycine I in the 10% solution resulted in no change of structure. Heating Polyglycine II in 10% calcium chloride, in which it would be soluble even at 140°C., also produced no change of structure, or no conversion to Polyglycine I. This seemed to confirm that at least in this case, the conversion of Polyglycine II into Polyglycine I takes place in solution.

When the Polyglycines were heated in glycerol, the results were similar to those for heating in water. The loss of polymer was much less in this case. The product obtained from Polyglycine II showed lines for glycine in its X-ray photograph, thus confirming that it is hydrolysed to glycine.

Heating in anhydrous formic acid converted Polyglycine II, partially, into Polyglycine I. Polyglycine I was unchanged by heating in this medium. It was found that the Polyglycine II was very readily attacked by the formic acid, whereas the Polyglycine I was not so readily attacked. This again would be in agreement with Polyglycine II being the metastable, and therefore more reactive form of Polyglycine.

THE STRUCTURES OF POLYGLYCINE I AND POLYGLYCINE II.

Polyglycine I.-

The experimental work has shown that it is extremely difficult to obtain pure, well crystallised samples of Polyglycine I. The best sample of pure Polyglycine I, was obtained by the polymerisation of diketopiperazine in water(reference.- Exp. Sec. 7). This material was obtained as ill defined micro crystals, showing no distinct crystal shapes or crystal cleavages. This material did however show a very sharp and distinct X-ray diffractometer trace. The best defined crystals of Polyglycine I were obtained from the polymerisation of glycine in phenol(reference.- Exp, Sec. 4.). The material showed only rectangular cleavage, and no distinct crystal shapes, under the electron microscope(reference.- Exp. Sec. 14.). The X-ray diffractometer trace for this material was not so well defined as for the above sample. The remaining preparations of Polyglycine I were shown to be impure, in that they contained some Polyglycine II. Some of this Polyglycine II impurity could be extracted by hot water. This material was shown to be a low molecular weight peptide showing the Polyglycine II structure. It was found that it was possible to precipitate

a low molecular weight form of Polyglycine I also. This material was obtained from the polymerisation of glycine in phosphoric acid in the open air (reference.-Exp. Sec. 8.).

The Polyglycine I obtained from the polymerisation of diketopiperazine in water, has been examined in detail. The diffractometer has shown that the diffraction peaks, are in fact more complicated than has been observed hitherto by the use of powder cameras. This is due to the greater peak resolving power of the diffractometer. The most significant result from this investigation, was the distinct difference in the position of one of the strong peaks, when compared with the results obtained from the photographs. The peak appeared consistently at 13° 8'(3.39A.)(v.s.), the value given by Astbury et alia[8,9] and by Bamford et alia[17], and Meyer and Go[92], was 12° 55'(3.45 A.). Some of the other peaks which had hitherto been thought to be finite, did in fact possess a fine structure.

Several structures have been proposed for Polyglycine I, but none have been definitely confirmed. The first structure was proposed by Astbury et alia[9] as being orthorhombic. This structure was later modified, Astbury[8], to a monoclinic cell.

The X-ray results which have been obtained here cast considerable doubt on this structure. The unit cell is certainly not orthorhombic, this can be readily shown. Neither does it correspond to a monoclinic cell, based on a modified Astbury cell. Reflections of the type h0l, could be made to correspond reasonably well to the modified monoclinic cell, but the agreement broke down completely for reflections of the type hkl. This suggested that the unit cell was triclinic. Pauling and Corey[97] suggested an anti parallel pleated sheet structure, with an ab-plane essentially as proposed by Astbury. The fibre identity period was proposed as 6.96A. to 7.0A.. Attempts were made to fit either of these limiting values for the fibre identity period into the X-ray diagram as a 020 reflection, resulted in values of θ_{020} which were much too low. A better figure for the fibre identity period was 6.79A.. This value is very low, the normal value for small peptides is about 7.23A.. This then makes $\theta_{020} = 13^{\circ}\ 8'$(v.s.), assuming a monoclinic cell. This did not give values for θ, for reflections involving k, which could be made to agree with the observed values.

It is concluded from this work that the structure has not been solved. This work cannot be further

extended by Powder photography, since the X-ray pattern is too complicated for solution. It is suggested that an attempt be made to obtain single crystals of Polyglycine I. This could possibly be done by recrystallisation of the hot water soluble Polyglycine I obtained from the polymerisation of glycine in phosphoric acid. This new low molecular weight Polyglycine I should be investigated in its own right in any case.

Polyglycine II.-

This material was obtained in much better defined crystalline form than Polyglycine II. The microcrystals were obtained by the repeated reprecipitation of the peptide, from solutions in saturated aqueous calcium chloride, by water. The product consisted of thin hexagonal leaflets, under the electron microscope. The several stages by which these platelets were obtained, whereby the crystals changed their shape quite radically, have also been observed under the electron microscope. The normal precipitated Polyglycine II is present in the "puff-ball" form. These"puff-balls" have been shown to disintegrate completely on conversion to Polyglycine I, no large particles of Polyglycine I were found. It has been found that in all cases the Polyglycine I has always been obtained as small

irregular particles. This indicates that the change of crystalline form involves a large change of the shape and or the dimensions of the unit cell of the Polyglycine II. A substantial proportion of the low molecular weight, water soluble Polyglycine II was obtained from the polymerisation of glycine in phosphoric acid in the open. This material could be recrystallised from hot water. The microcrystals obtained, were shown to be very thin, when compared with the above material, hexagonal leaflets, showing very distinct hexagonal growth steps and dislocations. The X-ray pattern of this material confirmed that it was Polyglycine II. This new material was undoubtedly the material studied by Meggy and Sikorski[91].

The electron diffraction photographs which have been obtained using this material, have been of little use in elucidating the structure of the Polyglycine II. The thin hexagons and the thicker hexagons would not give electron diffraction patterns. The orientation of the hexagons was in the plane of the grid, at right angles to the electron beam. The portions of the polymer which could be induced to give spot diffraction patterns were not recognisable as portions of the crystals. The fact that the Polyglycine II, whether of low or high molecular weight, is present as hexagonal plates

supports the hexagonal structure proposed by Crick and Rich[39]. The fact that no electron diffraction photographs could be obtained with the crystals in the given orientation, does not however support this structure.

The X-ray diffractometer traces of the material obtained from the reprecipitations from calcium chloride solution, has been examined in detail. The short 5 inches per hour chart traces showed good agreement with the Crick and Rich proposed structure. The 30 inches per hour traces showed that the peaks which had previously been considered as single peaks, were in fact multiple peaks in many cases, e,g. at $\theta = \approx 11^{\circ}$ and $\approx 14^{\circ}$. The multiple peaks were not due to statistical error, or variation of the X-ray tube output, since they were obtained consistently on several traces. It has been shown that although the Crick and Rich structure can account for some of the lines observed, it leaves many lines unaccounted for. The dimensions of the hexagonal cell have been slightly changed without producing any better agreement with the observed lines. The crystals do at least show a hexagonal cross section in the plane containing two of the axes of the unit cell. Crick and Rich stated that the proposed structure was only the simplest member of a whole family of related

structures. Thus it is possible that Polyglycine II is not homogeneous, but is a mixture of two or more closely related crystalline solid phases. The examination of possible unit cell sizes has shown that if this were so, the possible structures could not be hexagonal. Although they must be closely related since the material can be crystallised without change of the X-ray pattern. A critical comparison with the observed diffraction trace for the pentaglycylglycine(G_6) showed distinct similarities of basic structure. The material considered here cannot contain any pentaglycylglycine since this would be soluble in diluted calcium chloride solution. It has been suggested by Crick in a private communication, that the structure might deviate from that proposed if the polymer chains ran both up and down, in either a random or regular arrangement throughout the structure. The regular arrangement of the chains running up and down the structure of the unit cell would be expected to produce some extra spacings.

The remaining explanation is that the structure is a distorted hexagonal arrangement, and it belongs to the triclinic system. Attempts have been made, without success, to vary the angles between the a,b, and c axes of the crystals, no better agreement was

obtained with the observed lines. This method had only a slight chance of success.

The conclusions to be drawn from this work are that the Crick and Rich structure cannot be confirmed. The only method for solving the structure, is to prepare single crystals specimens. This could be done using the low molecular weight, hot water soluble material. The preparation of single crystals of a suitable size for analysis should not be very difficult. If this approach fails it should be even easier to prepare single crystals of the pentaglycylglycine and determine the structure of this material. It may then be possible to interpolate the structure of Polyglycine II. The pentaglycyl-glycine structure may in fact be a limiting structure, above which all the the higher polymers take up the polyglycine II form. In other words the unit cell of the structure is determined by the size of the glycine residue and not by the size of the molecule as a whole.

THE X-RAY INVESTIGATION OF THE LOW MOLECULAR WEIGHT PEPTIDES OF GLYCINE.

A search of the literature revealed that little work had been done on the structures of the low molecular weight peptides of glycine, other than

glycylglycine and diketopiperazine. An examination of the X-ray photographs showed that certain diffraction lines were common to more than one peptide pattern. Attempts were made to determine the structures of some of the lower peptides by making use of the Ito method. The X-ray patterns were very complicated, and the lines showed extensive overlapping of one another. The structures were not capable of solution from the X-ray powder photographs, showing that the crystals were of low symmetry. The X-ray powder diffractometer produced traces showing better resolution of the overlapping peaks. The charts showed much more detail than the photographs and could be more readily compared. The fullest possible set of X-ray data for each polymer specimen has been included to serve as comparison charts for future workers. Considerable difficulty was experienced in obtaining pure samples of some of the peptides, e.g. glycylglycine methyl ester hydrochloride. No recorded data could be found on the preparation of this particular peptide ester hydrochloride.

Diglycylglycine was prepared according to the method of Abderhalden[1]. The product was shown by Bernal[26] to be a hydrate of diglycylglycine, he also

showed that the unit cell for the material was orthorhombic, and gave the unit cell dimensions. The material which was obtained for this work was certainly not hydrated. The X-ray diffractometer trace showed that the unit cell was not orthorhombic. A determination of the degree of polymerisation of the material gave a value of 3. A chromatogram showed that the material was homogeneous. The diffraction pattern showed a distinct systematic absence for odd reflections of the type h00. There is little doubt that this material is diglycylglycine. The crystal structure of this material is quite different from that of the material investigated by Bernal. It was found to be too difficult to determine the unit cell for this new structure. This new form of diglycylglycine does require further investigation, and a critical comparison with the Bernal material should be carried out. The methyl and ethyl ester hydrochlorides of diglycylglycine have also been studied. The powder diffraction traces were again too complicated, and the crystals of too low a symmetry, for the solution to be carried out by the Ito method. Attempts to obtain single crystals of these compounds from solutions in aqueous ethanol, produced good crystals, but analysis showed that they had been

extensively hydrolysed in solution, leaving only about 35% of the ester hydrochloride. The melting points of these materials were unchanged on recrystallisation. Thus it is assumed that the ester hydrochloride and the hydrolysis products are capable of forming mixed crystals and so no change in melting point would occur. Any future work on the preparation of single crystals of these compounds should take this effect into account. The ester hydrochlorides are by far the best defined crystalline compounds of the lower peptides, and since they can form mixed crystals with the products of hydrolysis, they must be of closely related crystal structure. The powder diffractometer traces for the ethyl and methyl esters of diglycylglycine showed low Bragg angle spacings, $\theta = 3^{\circ}$(approx.), giving spacings of 14 to 16 A.. These spacings are repeated at higher orders. A reflection also occurs in both patterns at 2.09A., a reflection at 2.08A. was recorded for Polyglycine II. This reflection did not appear in the trace for diglycylglycine.

The powder diffraction trace for triglycylglycine showed no low angle peaks, the first peak appeared at 9° 12'(4.82A.). The trace showed four prominent peaks. The peak at 13° 21', also occurred in the trace for a low molecular weight product obtained

from the polymerisation of glycine in phosphoric acid by water washing the product. The remainder of the peaks on the two traces were entirely different. The triglycylglycine trace showed only first and second order peaks, and no peak occurred at 2.08A.. The trace differed entirely from that of the higher peptides.

Pentaglycylglycine gave a powder diffraction trace which showed strong similarities to that for Polyglycine II, strong peaks occurred coincident with the strong Polyglycine II peaks. This material also showed traces of ill defined low angle peaks. The more accurate results shown in Exp. Sec. 17., showed that many of the peaks of low angle, were present in higher orders; reflections were shown up to the tenth order. Attempts were made to interpret this pattern in terms of an hexagonal structure without success. It was possible to determine two of the main spacings but the third spacing is not so well defined. The structure of Pentaglycylglycine is certainly no simpler than that of Polyglycine II. The two structures are however closely related. The ease with which pentaglycylglycine can be recrystallised suggests that this material could be more easily obtained as single crystals than the hot water soluble Polyglycine II. The investigation of this

material would be of interest because of the low Bragg angle spacings, and because of the close relationship with Polyglycine II. This close structural relationship, prompts the suggestion, that the low molecular weight peptides, of degrees of polymerisation between say 6 and 10, tend to crystallise in the Polyglycine II structure, this tendency may be enhanced when the peptides are not homogeneous. An impure sample of pentaglycylglycine methyl ester hydrochloride, contaminated with some pentaglycylglycine, was also prepared. The diffractometer trace of this material showed that the ester hydrochloride gave a strong low Bragg angle reflections at 2° 43'(16.25A.). This spacing is not shown by pentaglycylglycine, and is more intense than the low angle reflections shown by pentaglycylglycine.

The presence of low angle spacings in the diffractometer traces of the benzoylpentaglycyl-glycine, and the product of polymerisation of glycine in phosphoric acid, have been detected. These samples are the first observed specimens of the polymers of glycine to give such low angle spacings. The unit cells must either be very large or otherwise, the structures exhibit a kind of superstructure. The low angle peaks which have been obtained in this work are too strong to be neglected.

and are repeated to very high orders, thus they must be geniune reflections. The apparatus used for this investigation was not designed for accurate use at very low angles. It is suggested that further investigations should be undertaken to see if there are any more very low angle spacings, and to obtain the more accurate results required for a full investigation of the observed low angle reflections.

The benzoyl pentaglycylglycine referred to above was prepared by the direct polymerisation of the benzoyl glycine and glycine in a melt. The work of Curtius and Benrath[40], suggested that this reaction was not possible. The material obtained showed a diffraction patterns closely resembling that for Polyglycine II. The pattern also showed one line which coincided with the strongest line in the pattern for benzoyl glycine. Similar polymerisation reactions carried out in sealed tubes produced no benzoyl pentaglycylglycine. It was found that the reaction mixture contained no solid glycine at the reaction temperature, thus the presence of benzoyl glycine in the reaction mixture, had increased the solubility of the glycine. Some Polyglycine II was obtained when the molar ratio of glycine to benzoyl glycine was increased, this polymer was later found to contain traces of Polyglycine I as well. Similar

results were obtained for the polymerisation of glycine and phthalyl glycine in sealed tubes. The acidity of the benzoyl and phthalyl derivatives seemed to aid the formation of polymer, but the increased solubility of the glycine, restricted the polymer formed to Polyglycine II, only. The hydrolysis of the benzoyl and phthalyl derivatives was not as extensive as was expected from thermodynamics.

THE DYEING OF THE POLYGLYCINES.

The dyeing of the Polyglycine I and II by Orange II dye, did not affect the structure of either polymer. No change was observed in the peak positions or intensities of the dyed polymer diffractometer traces. No lines were observed for the dye diffraction pattern. Thus the dye did not take up a regular arrangement within the crystal structures of the polymers, neither did it disturb the structures of the polymers. Mechanical mixtures of the Polyglycines and Orange II were prepared to show the lowest concentration at which the dye lines were visible in the diffractometer trace. The minimum concentration of dye necessary was 15% by weight. The X-ray lines for the dye were just visible at this concentration. Thus since the dyed polymers contained more than 25% dye , the X-ray lines for this concentration, would have appeared if the

dye molecules had been present in a regular arrangement. The Solution remaining after dyeing Polyglycine II obtained from zinc chloride, and centrifuging off the dyed polymer, was allowed to stand. Orange hair like crystals were deposited from this solution, This deposit was soluble in the supernatant solution, on dilution with water, and warming, but were reformed on cooling. The X-ray diffractometer trace for this material, showed lines for Polyglycine II, together with many other weak lines, some of which corresponded to Orange II lines. Only one of the strong Orange II lines was present as a strong line. This material is most probably a complex compound of the dye and low molecular weight Polyglycine II.

THE DETERMINATION OF THE CALCIUM AND ZINC CONTENTS OF PRECIPITATED POLYGLYCINE II.

The Polyglycine II precipitated from solutions in calcium chloride solutions, has been shown to contain calcium bound to one polymer molecule in nine polymer molecules. The calcium is thought to be present as the calcium salt. The remaining polymer is present as the acid. Similar results were shown for the Polyglycine II precipitated from 70% aqueous zinc chloride solution. One polymer molecule in every three or four was found to be

present as the zinc salt. Thus some kind of Donnan membrane effect is operative, so that the Polyglycine is capable of retaining ions from the solution.

THE CRYSTAL STRUCTURE OF DIGLYCINE HYDROCHLORIDE. (APPENDIX 2.).

A single crystal investigation of an old sample of glycylglycine ethyl ester hydrochloride, revealed that it had in fact been hydrolysed to give substantially diglycine hydrochloride. The structure of this compound has been investigated by Buerger and Hahn[30], but the results which have been obtained do at least confirm the allocation of the unit cell and the space group proposed by these workers. The melting point of this material was identical with that of glycylglycine ethyl ester hydrochloride, and the material was chromatographically pure. The melting point had remained the same as the ester hydrochloride melting point because of the formation of mixed crystals, as was observed in the case of diglycylglycine methyl ester hydrochloride. The single chromatogram spot was due to glycine only. This again confirms the ease with which the ester hydrochlorides may be hydrolysed, and underlines the trap into which one can fall when examining these compounds.

THE ABSORPTION OF COBALT BY THE POLYGLYCINES.

(APPENDIX 2.)

This work was undertaken jointly with Dr. A. B. Meggy and Dr. B. L. Tonge. My own part in the investigation was restricted to the production and interpretation of the X-ray powder photographs of the complexed Polyglycines. The results showed that the absorbed cobalt did not take up a regular arrangement in the structures of either Polyglycine I or II. No trace of any X-ray lines due to cobalt was observed. The X-ray pattern of the Polyglycines was also undisturbed, on complexing with the cobalt. These results are comparable with those obtained by dyeing the Polyglycines with Orange II.

RECOMMENDATIONS FOR FUTURE WORK.

1) Preparation of single crystals of the low molecular weight Polyglycine II, obtained from the products of the polymerisation of glycine in phosphoric acid. This should be possible using the method of separation given in this work.

2) Preparation of single crystals of pentaglycylglycine. This again should be easily possible in view of the solubility of the peptide.

3) A single crystal examination of the single crystals of hot water soluble Polyglycine II, and of pentaglycylglycine.

4) An electron diffraction investigation of the microcrystals of Polyglycine I and Polyglycine II should be carried out. Crystals of both the hot water soluble forms and the water insoluble forms of both polyglycines should be examined. The successful production of spot diffraction patterns would be of great help in elucidating the structures of the polyglycines.

5) The polymerisation of glycine in phosphoric acid may yield small quantities of higher polymers than the Polyglycines used in this work. Small quantities of this material have been obtained.

6) The investigation of the polymerisation of diketopiperazine in water has shown that certain

of the diketopiperazine X-ray lines disappeared before the others during the reaction. A thorough investigation of this polymerisation may shed light on the mechanism of the polymerisation of diketopiperazine, and on the structure of Polyglycine II.

7) The polymers which have been obtained are by no means homogeneous as regards molecular chain length. An attempt should be made to fractionate the polymerisation products. It is difficult to obtain suitable solvents for this process; a possible method would be an adaptation of the Baker and Williams [32] method of fractionation, for the determination of molecular weight distributions. The solvent non solvent system could be aqueous calcium chloride and water solutions. The polymer would precipitate out readily onto the glass beads in the column, since it shows a pronounced tendency to stick firmly to glass on precipitation from solution. The polymer could then be eluted from the glass beads by the aqueous calcium chloride solution solvent. This method would provide only small quantities of peptides but these would be sufficient for X-ray powder photographs, electron microscopy, infra-red measurements, and chromatography.

8) Chromatography of the lower peptides using phenol and water(1:1) as the moving phase has been attempted, and has been shown to move the higher peptides, pentaglycylglycine and above. The chlorination method for developing the spots does not work for this system. An alternative method is to heat the paper strongly when the peptide spots will char. This will again aid the identification of polymer fractions.

9) The fractionation procedure described above, might possibly determine whether or not Polyglycine II consists of more than one phase.

10) The polymerisation of glycine in glycerol and the preparation of diketopiperazine has shown that side reactions occurred in addition to polymerisation. The side reactions leave a brown residue, this is mostly diketopiperazine together with some low molecular weight Polyglycine II, suspended in the glycerol or glycol. Also a brown material is left dissolved in the glycerol or glycol. It has been shown that this material could be partially extracted with acetone. The remainder of the colour could then be extracted with ether. The X-ray powder diffraction trace of the material extracted by acetone is given, but the experiment was not followed up. An investigation of this brown material

might help in determining the mechanism of the formation of diketopiperazine in this reaction. It might also lead to a method for improving the low yields of this reaction.

Appendix 1.

THE CRYSTAL STRUCTURE OF DIGLYCINE HYDROCHLORIDE.

An old sample of glycylglycine ethyl ester hydrochloride was used to determine its crystal structure. The material was recrystallised, the product obtained had a melting point of 182° C.. The melting point of glycylglycine ethyl ester hydrochloride is given in the literature as 181° to 182°C.. Hence it was assumed that the substance was glycylglycine ethyl ester hydrochloride. However in the course of the X-ray investigation, it was found that the dimensions of the unit cell gave a crystallographic density of 1.684 gm. per cc., whereas the measured density was found to be 1.595 gm. per cc., by the sink and float method, in carbon tetrachloride, together with a little iodobenzene. The substance appeared to be homogeneous from a paper chromatogram. The infra-red spectrum differed from that of glycine, as did the X-ray diffractometer traces. The total analysis and Sorenson titration showed that the substance was a molecular compound of glycine and glycine hydrochloride. This had been formed from glycylglycine ethyl ester hydrochloride by hydrolysis during storage. A full structural analysis of this compound has been reported by Buerger and Hahn[29,30].

However it was decided to complete the X-ray analysis of the space group and the unit cell for the structure.

An optical goniometrical study was carried out in order to determine the positions of the axes of the crystal, and to determine as much as possible about the symmetry of the crystal. The result of this investigation showed that the three axes were orthogonal, and that the long Y-axis was a twofold rotation axis. The majority of the crystals exhibited an elongated habit, favouring either the tetragonal or orthorhombic systems. It did not show any of the prism faces normally associated with the tetragonal system, as major faces. The major prism zone faces were those usually shown by the orthorhombic system.

SINGLE CRYSTAL X-RAY EXAMINATION.

A preliminary series of oscillation photographs was taken using several crystals. The best formed typical crystals were then selected for a detailed examination. The crystal system to which the crystals belonged, was determined by oscillation photographs taken for equal angles of oscillation on either side of each axis in turn. These photographs showed the diffraction spots to be symmetrically disposed about

each vertical oscillation axis in turn, each axis was at ninety degrees to the other. This showed that the directions chosen as axial directions were the true axial directions. A further examination of the photographs showed that in all three cases the distribution of the diffraction spots about the equatorial layer line was symmetrical with respect to both position and intensity. The axes were thus orthogenal, and since the layer line spacing for each axis was different the crystals belonged to the orthorhombic system.

DETERMINATION OF THE UNIT CELL.

Measurement of the distance between the layer lines on each oscillation photograph gave a value for the unit cell dimension in the direction used as the oscillation axis. The values obtained for the three axial directions were .-

a = 8.12 A. a^* = 0.19

b = 5.35 A. b^* = 0.288

c =17.9 A. c^* = 0.086

The ratio of .-

a : b: c = 1.52 : 1 : 3.35 .

This gave values accurate to about 1%. A more exact measurment was made by using the standard method of refinement. The values obtained above were used to draw a reciprocal lattice. Oscillation

photographs were obtained for oscillations of seven and a half degree angles on either side of each axis, i.e. the axis was set in the direction of the X-ray beam. The photograph obtained showed a symmetrical distribution of diffraction spots about the vertical axis. The distance between pairs of symmetrically placed spots on the zero layer line was measured. This was used to determine the Bragg angle θ^{o}, i.e. as if it were a powder photograph. The indices hkl. for each pair of spots was determined by plotting on the reciprocal lattice. Thus knowing the hkl values for the spot and its corresponding Bragg angle, it was possible to calculate the axial dimension, of the axis lying in the X-ray beam, for that particular value of θ. This was carried out for each axis in turn. Graphs were plotted for each axial direction of the values obtained for each axial dimension, against $\sin^{2}\theta$. For high values of θ this was taken as a straight line, and the graph extrapolated to $\theta = 90^{o}$. This gave the true values for the axial dimensions. The results .-

ACCURATE UNIT CELL DIMENSIONS.-

$a = 8.094 \pm 0.01$ A.

$b = 5.31 \pm 0.01$ A.

$c = 18.02 \pm 0.01$ A.

DETERMINATION OF THE SPACE GROUP.

A series of photographs were taken by oscillating the crystal about the Y and Z axes, as vertical oscillation axes, through angles of 15° at 10° intervals. These photographs were plotted on the reciprocal lattice. The intensities of each diffraction spot were graded by eye, and a reciprocal net drawn showing the intensities at each lattice point. This showed that reflections were systematically absent along the three axial directions, and only along these three directions. Thus the crystals belong to the space group $P2_12_12_1$, and show a two-fold screw axis along each of the axial directions. A calculation of the density of the crystals from the molecular weight of the substance, the volume of the unit cell, as determined above, and assuming that the unit cell contained four molecules, gave a value of d = 1.684 gm. per cc.. This compared with the practically determined density of 1.595 gm. per cc. THUS THE ORIGINAL MATERIAL COULD NOT POSSIBLY HAVE BEEN GLYCYLGLYCINE ETHYL ESTER HYDROCHLORIDE.

The material was homogeneous since it could be recrystallised wothout change of melting pointp and it was chromatographically pure. A reinvestigation of the material was carried out.

ANALYSIS.

The result of an elementary analysis.-

Carbon 25.52 %

Hydrogen 6.08 %

Nitrogen 15.02 %

Chlorine 18.82 %

Ethoxyl 4.55 % (this was reduced to 0.39 % on drying over calcium chloride in a desiccator.)

This did not agree with the calculated values for glycylglycine ethyl ester hydrochloride.

[GLYCYLGLYCINE ETHYL ESTER HYDROCHLORIDE.-

Carbon 36.65 %

Hydrogen 6.625 %

Nitrogen 14.25 %

Chlorine 17.82 %

Ethoxyl 22.9 %]

The observed nitrogen to chlorine ratiom of the material was 1.97, this would correspond to a peptide containing two units of glycine. A number of possible structures such as glycylglycine Hydrochloride and mixtures of this and the ethyl ester hydrochloride, or this and the ethyl and methyl ester hydrochlorides etc. were examined. The above values did not fit any of the mixtures, or glycylglycine hydrochloride alone, it would not fit compounds of glycylglycine

hydrochloride and ethanol or methanol either.

A chromatogram comparing the material with a number of other glycylglycine compounds and glycine showed that the material could not be separated from glycine. It ran parallel with a glycine control spot in a moving phase of butanol, acetic acid, and water, or in butanol, pyridine, and water, or in phenol and water. The infra-red spectra of glycine and the material were not the same, they differed markedly. It was then realised what had happened. The glycylglycine ethyl ester hydrochloride had become damp at some stage during storage, and had been hydrolysed to ethanol, glycine and hydrochloric acid. On recrystallisation of the material, the mixture was of the correct proportions to give the compound described by Frost[60] and Kraut and Hartmann[77]; namely diglycine hydrochloride, $2(C_2H_5NO_2).HCl$.. The theoretical figures for the analysis of this material were.-

DIGLYCINE HYDROCHLORIDE.-

Carbon	25.37 %
Hydrogen	5.91 %
Nitrogen	15.0 %
Chlorine	19.05 %

Except for the hydrogen figure, these values all agree within one percent with the values obtained

for the unknown material. Also the nitrogen to chlorine ratio would be two as for a dipeptide.

The crystal structure of diglycine hydrochloride has been investigated by Buerger and Hahn[29,30]. They showed that the crystals had orthorhombic symmetry, and systematic abscences characteristic of the space group $P2_12_12_1$. The unit cell had the following dimensions.-

a = 8.15 A.	Unknown	a = 8.094 A.
b = 18.03 A.		c = 18.02 A.
c = 5.34 A.		b = 5.31 A.

Measured density .-

d = 1.581 gm. per ml.	d = 1.595 gm. per ml. at 23°C.

Calculated density assuming 4 moles per unit cell was d = 1.579 gm. per ml..

The material was therefore without doubt identical with diglycine hydrochloride. If the crystal was allowed to stand in air; a series of oscillation photographs showed that it underwent a continuous lattice expansion, presumably absorbing water. As this lattice expansion continued, it was found that it was no longer possible to obtain symmetrical oscillation photographs. Thus for oscillations about the vertical y axis, with the former x -axis lying along the X-ray beam, it was found that the

diffraction spots were no longer symmetrically distributed about the vertical axis of the photograph. Thus the position of this axis had changed, in that it was no longer at right angles to the other two axes. The symmetry of the photograph about the equatorial layer line had also been destroyed. The structure seemed to be taking up a monoclinic or triclinic structure. This expansion of the lattice was still continuing months later.

Diffractometer traces of powder specimens of the unknown material are shown together with similar traces for related compounds on the Chart 12.

THE RECRYSTALLISATION OF THE METHYL AND ETHYL ESTERS OF LOWER GLYCINE PEPTIDES.

It was thought that it was possible to recrystallise the methyl and ethyl esters of glycylglycine and diglycylglycine from solutions in aqueous alcohols. It has been found that the ester hydrochlorides are extensively hydrolysed in this solvent. The methyl ester hydrochloride of diglycylglycine gave a product which contained only 33% of the ester hydrochloride on recrystallisation. Similar results have been obtained for the ethyl ester hydrochloride, and for the corresponding glycylglycine compounds. The esters are very easily hydrolysed to the peptides in solution , and on recrystallisation the peptides and ester hydrochlorides seemed to form mixed crystals. Hence no depression of the melting point of the recrystallised product was observed.

Appendix 2.

(*Reprinted from Nature, Vol. 198, No. 4885, pp. 1084–1085, June 15, 1963*)

Cobalt Complexes of Polyglycines

THE binding of cobalt to proteins and polypeptides has been shown to occur in a number of cases, particularly under conditions of high alkalinity, where ligation occurs with amide groups in addition to the terminal functions[1].

In the course of our investigations on the chemical reactions of the polyglycines I and II[2], we noticed a difference in the ability of these isomers to form complexes with metals, particularly cobalt. The structures of the polyglycines[3] differ in that polyglycine I (prepared from piperazine-2,5-dione and water at 180° for 6 h) is an almost fully extended β-polypeptide, whereas polyglycine II (prepared by precipitating polyglycine I from a solution in a strong electrolyte) is a folded structure having a 3-fold screw axis, the chains being packed in a hexagonal array. In both structures a large proportion of the terminal groups would be available for co-ordination with a cobalt ion.

The uptake of cobalt from solution in excess of concentrated ammonium hydroxide was followed using a radioactive tracer technique using cobalt-60. Polyglycine I (degree of polymerization (DP) about 12) absorbed 3·2 moles cobalt per mole polymer, whereas polyglycine II (DP about 12) only absorbed 1·1 moles cobalt per mole polymer. From the shape of the absorption isotherms, the accessibility of cobalt into the polyglycine II lattice appears to be limited by electrical or diffusion phenomena characteristic of the uptake of dye by a natural or synthetic polypeptide. No irregularities were found in the case of polyglycine I.

These results suggest that a cobalt (III) ion, possibly also co-ordinated by ammonia molecules, cannot be readily accommodated by the three-fold screw axis, but can be fitted into the more extended polyglycine I structure. X-ray diffraction photographs of the polymers and their respective complexes show a small lengthening of the unit spacing only for the conversion of polyglycine I into the cobalt complex.

Meggy and Sims[4] showed that polyglycine I absorbed orange II equivalent to only 42 per cent of the total terminal amino-groups, whereas polyglycine II absorbed the theoretical amount of dye on the terminal residues. In neither case was the X-ray diffraction pattern disturbed on absorption of dye. Earlier, Bamford and his co-workers[5] had commented on the ready absorption of certain dyes on the amide groups of a β-polypeptide

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INDEX TO THE X-RAY DIFFRACTOMETER CHARTS.

The chart traces are indexed by numbering, from left to right across the sheet, and from the top to the bottom of the sheet.

CHART No. 1.

1) The product from the melt of glycine(10 g.) and benzoyl glycine(10.5 g.), heated in the open. The product crystallised from a boiling water extract of reaction mixture, on filtration and cooling.
2) This sample was deposited from the cold filtrate obtained after the removal of sample 1) above, and allowing the filtrate to stand overnight.
3) The bulk residue from the polymerisation in the melt of glycine(10 g.) and benzoyl glycine(10.5 g.).1 st. prep'n.
4) A sample of the benzoyl glycine used above.
5) The bulk residue from the second preparation of the glycine and benzoyl glycine polymerisation product.

CHART No. 2.

1) Product from the polymerisation of glycine(5 g.) and phenol/water(5 ml.)[the upper phase], heated in a sealed tube at 140°C., for four weeks.
2) Product from heating glycine(5 g.) alone in a sealed tube, for one week at 180°C.

CHART No. 2 (continued).

3) The product from the polymerisation of glycine(10 g.), when heated in diphenylamine, in a sealed tube for 2 weeks, at 150°C.

4) Glycine(5 g.) was heated with water(1 ml.) in a sealed tube at 160°C., for 48 hours. The insoluble residue was washed with water and alcohol, and centrifuged down and dried. This sample was taken from the bottom of the centrifuge tube.

5) Polyglycine I prepared by the polymerisation of diketopiperazine(10 g.) in water(5 ml.), by heating in a sealed tube for 6 hours at 170°C.

6) See trace 4) above. This sample was taken from the top of the centrifuge tube.

CHART No. 3.

1) The polymerisation of glycine(5 g.) in hydrochloric acid(5 ml. conc.), heated sealed at 140°C. for 7 hours. This sample was taken from the top portion of the carius tube, which had been totally liquid at 140°C..

2) See trace 1) above. This sample was taken from the bottom of the carius tube, which had contained solid at 140°C.

3) The same reaction mixture as above was heated for 11 hours. This sample was taken from the upper liquid, at 140 C., portion of the carius tube.

CHART No. 3 (continued)

4) See trace 3) above, the sample was taken from the lower portion of the carius tube, which contained solid at 140°C.

5) The same reaction mixture was heated for 14 hours. This sample was taken from the upper portion of the cooled reaction mixture.

6) See trace 5) above, this sample was taken from the lower portion of the reaction mixture.

7) The same reaction mixture was heated for 24 hours at 140°C. This sample was taken from the washed solid extracted from the total reaction mixture.

8) The same reaction mixture was heated at 200°C. for 24 hours. This sample was the bulk low molecular weight peptides of glycine, which were soluble in hot water, but could be precipitated from the cold solution by adding ethanol.

CHART No. 4.

1) The bulk washed product from the polymerisation of glycine (40 g.) in concentrated hydrochloric acid(16 ml.) by heating in a sealed tube at 150°C. for 24 hours.

2) Polyglycine II, prepared by repeated reprecipitation from solutions in saturated aqueous calcium chloride solution, by the addition of water.

CHART No. 4(continued).

3) Polyglycine II(donuts), prepared by repeated precipitation from solution in 70% aqueous zinc chloride solution, on the addition of water.

4) Polyglycine II(thick hexagonal microcrystals), from the repeated reprecipitation from solution in saturated aqueous calcium chloride soluion, by water.

5) Polyglycine, prepared by the precipitation of a 5% solution of Polyglycine I in 70% aqueous zinc chloride solution(5 ml.) by 60 ml. of industrial spirit, and washed with industrial spirit.

6) Polyglycine prepared by precipitating 5 ml. of 10% solution of Polyglycine I in 70% aqueous zinc chloride solution, by 60 ml. of industrial spirit.

7) Polyglycine, from the polymerisation of glycine(5g.) in concentrated hydrochloric acid(1ml.) in a sealed tube at 153°C. for 24 hours. The sample was taken from the top crust of the centrifuged water insoluble residue.

CHART No. 5.

1) Diketopiperazine(5 g.) and water(5 ml.) heated sealed at 160°C. for 4 hours. The sample was taken from the water and ethanol washed, water insoluble residue.

2) Diketopiperazine, prepared from glycine and glycol.

CHART No5. (continued)

3) Diketopiperazine and water(5 g. and 2½ ml.) heated sealed at 160°C. for 5 hours. The sample is taken from the alcohol and water washed residue.

4) Diketopiperazine(10 g.) and water(7 ml.) heated sealed for 6 hours at 160°C. The sample is taken from the washed insoluble residue.

5) Polyglycine, prepared by boiling carbenzoxy-glycine anhydride in pyridine.

6) Polyglycine, prepared from carbenzoxy-glycine anhydride polymerisation in the presence of water vapour, at room temperature.

CHART No. 6.

1) Analar glycine.(B.D.H.)

2) Glycine Hydrochloride, prepared by the Frost method.

3) Glycine Ethyl Ester Hydrochloride (Mpt. 145°C.).

4) Diglycine Hydrochloride, Frost method(Mpt. 186°C.).

5) Diketopiperazine, prepared from glycine and glycol.

6) Glycylglycine Hydrochloride Hydrate, (Mpt. 141°C.).

CHART No. 7.

1) Glycylglycine Hydrochloride hydrate (Mpt. 141°C.)

2) Glycylglycine Ethyl Ester Hydrochloride, prepared by the Schott, Larkin, Rockland and Dunn method, (Mpt. 185-186 °C.)

CHART No. 7.(continued)

3) Glycylglycine Methyl Ester Hydrochloride,(Mpt. 189°C.)

4) Diglycylglycine, recrystallised from ethanol/water, [Mpt. 246°C.(d.)].

5) Diglycylglycine Ethyl Ester Hydrochloride,(Mpt. 216°C.)

6) Diglycylglycine Methyl Ester Hydrochloride, (Mpt. 196°C).

CHART No. 8.

1) Triglycylglycine , [Mpt. 220 - 270°C(d)].

2) Pentaglycylglycine.

3) Pentaglycylglycine methyl ester hydrochloride.

4) Polyglycine II, repeated reprecipitation from solution in calcium chloride.

5) Polyglycine I, ex. diketopiperazine(10g.) heated in water(5 ml.), in a sealed tube at 170°C. for 6 hours.

CHART No. 9.

1) Polymerisation of glycine(30 g.) in concentrated phosphoric acid(16 ml.) heated in the open at 164°C. for 10 minutes. The sample was taken from the hot water washed insoluble residue.

2) See trace 1) above, the sample was taken from the solid which crystallised on cooling out of the first hot water extraction of the reaction mixture.

4) See trace 2) above, deposit from the filtered solution on standing overnight.

CHART No. 9 (continued).

5) A sample taken from the second hot water soluble fraction

5) See trace 4), solid extracted from the first hot water extraction solution, on adding excess ethanol.

6) See trace 3), solid extracted from the second hot water extraction solution, on adding alcohol.

CHART No. 10.

1) Polymerisation of glycine(20 g.) and concentrated phosphoric acid(20 ml.) heated in the open for 30 minutes at 170°C. A sample of the black water insoluble product.

2) See trace 1), leaflets deposited on cooling from the hot water extraction.

3) Hot water washed polymer from the polymerisation of glycine (40 g.) in phosphoric acid(5ml.), dried in air. The sample was taken from the grey bottom residue of the centrifuged product.

4) See Trace 3), sample taken from the dark top crust of the dried centrifuged product.

5) See trace 3), a ground unoriented sample of the hot water soluble portion of the product.

6) See trace 3), low molecular weight peptide from the second hot water extraction of the reaction mixture.

7) See trace 6), deposited on adding ethanol to the solution from the second extraction.

CHART No. 11.

1) Polymerisation of glycine(80 g.) in phosphoric acid (16 ml.) by heating in the open at 164°C. Sample from the residue on cooling the third hot water extract.

2) See trace 1) above, deposit obtained on treating the filtrate from above with excess ethanol.

3) See trace 1) above, solid deposited on cooling from the fourth hot water soluble extract, the sample was used as oriented by centrifuging down.

4) Polymerisation of glycine(20 g.) in phosphoric acid (5 ml.), by heating in a sealed tube at 170°C., for 21 hours. The sample is taken from the first recrystallisation of the hot water soluble fraction. This sample showed hexagonal leaflets under the electron microscope.

5) Polymerisation of glycine(20 g.) in phosphoric acid (5 ml.), by heating sealed at 157°C. for 24 hours, This sample was taken from the hot water soluble polymer.

6) See trace 5) above. This sample was taken from the bulk water washed polymer.

7) Polymerisation of glycine(5 g.) in phosphoric acid(1 ml.), by heating sealed at 147°C. for 24 hours. The reaction mixture was extracted with hot water, the polymer precipitated on cooling filtered off, and the solution allowed to stand, this sample was then deposited.

CHART No. 12.

1) This sample was thought to be glycylglycine ethyl ester hydrochloride, but was shown by the single crystal analysis to be totally converted to diglycine hydrochloride.
2) Hot water soluble Polyglycine II, obtained from the preparation of diketopiperazine, by the dimerisation of glycine in glycol.
3) Diglycine hydrochloride, prepared by the Frost method.
4) The sample was taken from the dried brown sludge obtained during the preparation of diketopiperazine, from glycine and glycol.
5) Glycylglycine ethyl ester hydrochloride, prepared by the method of Schott, Larkin, Rockland and Dunn.
6) Glycine hydrochloride, prepared by the method of Frost.

CHART No. 13.

This chart was one of several traces made for samples of Polyglycine II, reprecipitated from solutions in calcium chloride solution by water. The chart speed was 30 inches per hour. The numbers on the chart refer to this chart only. The numbers in the circle refer to the numbered peaks given in the list on page 8a(Exp. 16). The near horizontal line is an attempt to draw a mean background level.
