571. *Glycine Peptides. Part III.* The Absorption of Orange II by Polyglycine.*

By A. B. Meggy and D. Sims.

Polyglycine I, prepared from piperazine-2 : 5-dione and water, and polyglycine II, prepared by precipitating polyglycine I from calcium chloride solution, both absorb Orange II from aqueous solution. Polyglycine II absorbs Orange II equivalent to the total terminal amino-groups in the polymer. Polyglycine I absorbs only 42% of the theoretical amount; heating the polymer reduces this to 13%. Conversion into polyglycine II restores the absorption to 100% of theory, and the absorption by polyglycine II is not affected by heating of the polymer. The pK of the carboxyl group in polyglycine II is 2·9; that of the amide group, about 0. The affinity of the Orange II anion for polyglycine II is −6·3 kcal. mole compared with −4·6 for wool.

The absorption of acids and acid dyes by insoluble proteins and by nylon, has been studied by many workers.[1] Their results show that under suitable conditions one equivalent of acid is absorbed for each basic group in the protein, although in strongly acid media the amide groups also function as basic groups. The method has been extended to soluble proteins.[2] If acid dyes are used, the amount of acid absorbed can be readily estimated colorimetrically. Glycine polymers contain only one basic group, the terminal amino-group of the peptide chain, and if the amount of this group present in the polymer could be estimated from the absorption of an acid dye, it would provide a convenient method for estimating the equivalent weight and the number-average degree of polymerisation of the polymer. When attempts were made to apply the method, using the dye Orange II

* Part II, *J.*, 1953, 851.

[1] (*a*) Vickerstaff, "Physical Chemistry of Dyeing," 2nd Edn., Oliver & Boyd, London, 1954; Speakman and Elliot, "Symposium on Fibrous Proteins," Soc. Dyers and Colourists, Bradford, 1946, p. 116; (*b*) Vickerstaff and Lemin, *ibid.*, p. 128; (*c*) Meggy, *Trans. Faraday Soc.*, 1947, **43**, 502; (*d*) Steinhardt, Fugitt, and Harris, *J. Res. Nat. Bur. Stand.*, 1940, **25**, 519; 1941, **26**, 293; 1943, **30**, 123.

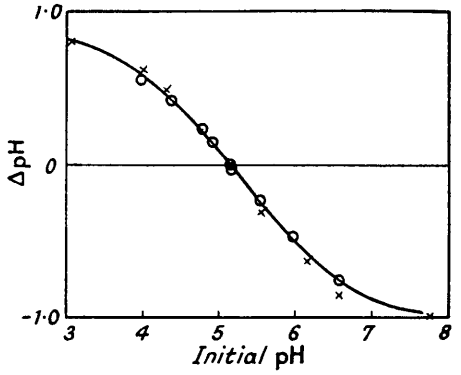
[2] Fraenkel-Conrat and Cooper, *J. Biol. Chem.*, 1944, **154**, 239.

(sulphanilic acid→2-naphthol; C.I. No. 156), anomalous results were obtained,[3] the amount absorbed being sometimes considerably less than 1 equivalent per amino-group. It was therefore decided to investigate more fully the absorption of Orange II by polyglycine.

Polyglycine was prepared by heating piperazine-2 : 5-dione with water at 160—180°. The product showed principal *X*-ray reflexions at 3·40 and 4·35 Å, showing that it was the type I polymer.[4] The isoionic point was determined by Vickerstaff and Lemin's method.[1b] The change in pH of the solution in equilibrium with the polymer on change of the electrolyte concentration by a known factor is plotted against the initial pH in Fig. 1. No change in pH is observed when the initial pH is 5·1. After washing with sodium acetate–acetic acid buffer of pH 5·1, the result is the same. The isoionic point of the polymer is therefore 5·1, in good agreement with the value for the lower peptides of glycine.[5]

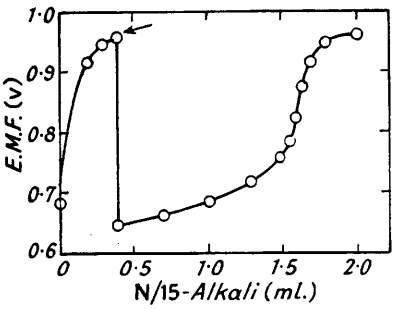
The following preparations of polyglycine were made. (*a*) Standard polymer. The polymer prepared at 160—180° was bulked and stirred with 0·1N-acetate buffer (pH 5·1) for 24 hr., then washed and dried. (*b*) Acid-washed polymer. Standard polymer was stirred with 0·1N-hydrochloric acid for 2 hr., washed, and dried. (*c*) Heated polymer. Standard polymer was heated in an air-oven at 105° for 10 days.

FIG. 1.



× *Before washing with buffer.* ○ *Washed with* 0·1N-*acetic acid–sodium acetate buffer* (pH 5·1).

FIG. 2.



Titration of the polymer in LiBr *solution.*

(*d*) Precipitated polymer. Standard polymer, dissolved in saturated calcium chloride solution, was poured into water at room temperature. (*e*) Heated and precipitated polymer. Heated polymer prepared as in (*c*) was precipitated as in (*d*). (*f*) Precipitated and heated polymer. Precipitated polymer prepared as in (*d*) was heated as in (*c*).

Preparations (*a*), (*b*), and (*c*) showed strong *X*-ray spacings at 3·40 and 4·35 Å, showing that they were all polyglycine I. Preparations (*d*), (*e*), and (*f*) showed a strong reflexion at 4·15 Å; these were all polyglycine II. It is apparent that heating in the dry state does not alter the crystal type.

The degree of polymerisation of the polymers was determined by titration in lithium bromide solution in the presence of formaldehyde, according to Sluyterman and Labruyere's method.[6] Calcium chloride solution could not be used as a solvent, because the glass electrode did not respond to changes in acidity therein; apparently the potential was determined by the calcium ions. An antimony electrode was also inoperative in both lithium bromide and calcium chloride solutions. The reference electrode was silver–silver bromide in saturated lithium bromide used as an external electrode. An internal electrode could not be used because of the reducing action of formaldehyde on silver bromide.

A typical titration curve is shown in Fig. 2. The addition of alkali to the solution of

[3] Meggy, *J.*, 1953, 851.
[4] Meyer and Go, *Helv. Chim. Acta*, 1934, **17**, 1488; Bamford, Brown, Cant, Elliot, Hanby, and Malcom, *Nature*, 1955, **176**, 396.
[5] Cohn and Edsall, "Proteins, Amino Acids, and Peptides," Rheinhold, New York, 1943; Glasstone and Hammel, *J. Amer. Chem. Soc.*, 1941, **63**, 243.
[6] Sluyterman and Labruyere, *Rec. Trav. chim.*, 1954, **73**, 347.

the polymer in lithium bromide solution caused a sharp rise in E.M.F. On addition of formaldehyde, at the point indicated by the arrow, the E.M.F. fell to below its original value. Further addition of alkali gave a typical sigmoid curve. The end point was taken as that at which the slope was a maximum. The total titre was the amount of alkali added, less a correction for the acid present in the formaldehyde, which was found by a blank titration. No correction was necessary for the lithium bromide solution.

Sluyterman and Labruyere,[6] using polymers prepared from *N*-anhydrocarboxyamino-acids, found an initial acidity which could be titrated in the absence of formaldehyde. They concluded that there was an excess of carboxyl over amino-groups in their polymers, which they attributed to the formation of hydantoin rings at the amino-end of the peptide chains. The sharp rise in E.M.F. with the first addition of alkali, as shown in Fig. 2, indicates that the solution of polymer in lithium bromide solution shows no initial acidity, before the addition of formaldehyde. The shape of the first part of the curve in Fig. 2 is practically identical with that obtained when lithium bromide solution is titrated with alkali. It seems, therefore, that the polymers obtained from piperazine-2 : 5-dione have equal numbers of carboxyl and amino-end-groups; this is supported by the results obtained for the combination with Orange II.

When standard polymer was dissolved in lithium bromide solution, and aliquot parts were titrated immediately on dissolution (about 30 min. after mixing), after 5 hr. and after 5 days, consumption of alkali was 1·38, 1·41, and 1·38 milliequiv./g. respectively. Therefore the polymer was stable in solution, and it is unlikely that it degraded during dissolution.

The six polymer preparations were titrated; the results are given in Table 1. Only acid-washed polymer showed initial acidity. The formol acidity was the same in all cases, within the limits of error, and corresponded to a number-average degree of polymerization (D.P.) of 12·3. It may be assumed that the various treatments have not altered this.

TABLE 1.

Prepn.	Type	Acidity (milliequiv./g.) Initial	Formol	Orange II (milliequiv./g.)
(*a*) Standard	I	0	1·39	0·59
(*b*) Acid-washed	I	0·88	1·40	—
(*c*) Heated	I	0	1·40	0·18
(*d*) Pptd.	II	0	1·39	1·45
(*e*) Heated and pptd.	II	0	1·40	—
(*f*) Pptd. and heated	II	0	1·39	—

Combination of the standard polymer (*a*), the heated polymer (*c*), and the precipitated polymer (*d*) with the dye Orange II in aqueous solution was studied in detail. The combination at 60°, as the free acid, is illustrated in Fig. 3. The curves show the widely different combining power in the three cases. If the dye combined with the terminal amino-groups of the peptide chains, and with these only, dye combination should reach a limit of about 1·40 milliequiv./g. in each case. Although two of the curves show an inflexion, none tends to a maximum. This is because at the higher concentrations dye is also absorbed on the amide groups. This effect is negligible at low concentrations of dye in the aqueous phase, so extrapolating the curves at low concentrations of dye to high concentrations reveals the limiting value for combination with the terminal amino-group. This is done [1b] by plotting $1/(\text{dye absorbed})$ against $1/\sqrt{[(H^+)(D^-)]}$, where (H^+) is the activity of hydrogen ions and (D^-) that of dye anions, as in Fig. 4. Then combination of Orange II with the terminal amino-groups reaches a limiting value of 0·59 milliequiv./g. for standard polymer (*a*), 0·18 for heated polymer (*c*), and 1·45 for precipitated polymer (*d*). Only with the precipitated polymer (*d*) does the amount of Orange II combined correspond to the total number of terminal amino-groups in the polymer; for the standard polymer (*a*) and the heated polymer (*c*) the amount of Orange II combined represents about 42% and 13% of the terminal amino-groups respectively.

The combination of another dye acid, Cardinal Red J, (4-aminonaphthalene-1-sulphonic acid→2-naphthol; C.I. No. 176) with standard polymer (*a*) is shown in Fig. 5, and the

reciprocal plot is shown in Fig. 4. For this dye the combining power is 0·61 milliequiv./g., compared with 0·59 for Orange II. The difference is not significant, and it seems that the fractional result is due to a property of the polymer, and not of the dyes.

Complete absorption curves were not prepared for the heated and precipitated polymer (*e*), or the precipitated and heated polymer (f), but a few determinations of absorption under identical conditions were made for polymers (*d*), (*e*), and (*f*). No significant differences were found between them; *e.g.*, in a particular experiment, the absorptions were 1·28 milliequiv./g. for (*d*), 1·24 for (*e*), and 1·26 for (*f*). It appears that the absorption curve for (*e*) and (*f*) would be identical with that for (*d*).

Preparations (*d*), (*e*), and (*f*) are all polyglycine II, having a hexagonal crystal structure.[7] It appears that for this form of polyglycine all the amino-groups are capable of combining with dye acids, irrespective of the method of preparation. Preparations (*a*) and (*c*) are polyglycine I, having a monoclinic crystal structure [8] similar to Nylon 6 : 6; in this form of polyglycine only a fraction of the amino-groups are capable of combining with dye acids. The number of groups available depends on the method of preparation, and is reduced when the polymer is heated.

FIG. 3. *Combination of Orange II with standard polymer (a), precipitated polymer (d), and heated polymer (c).*

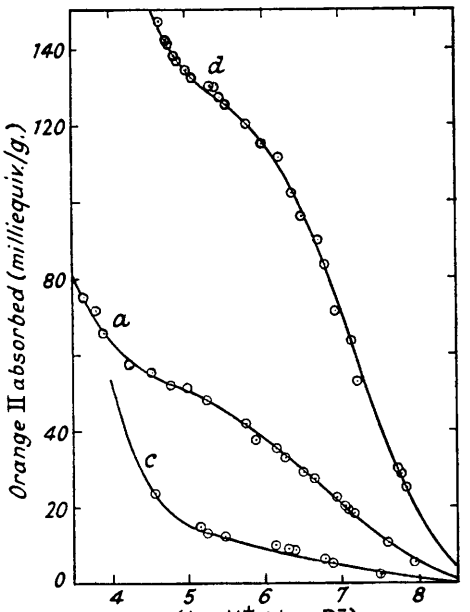
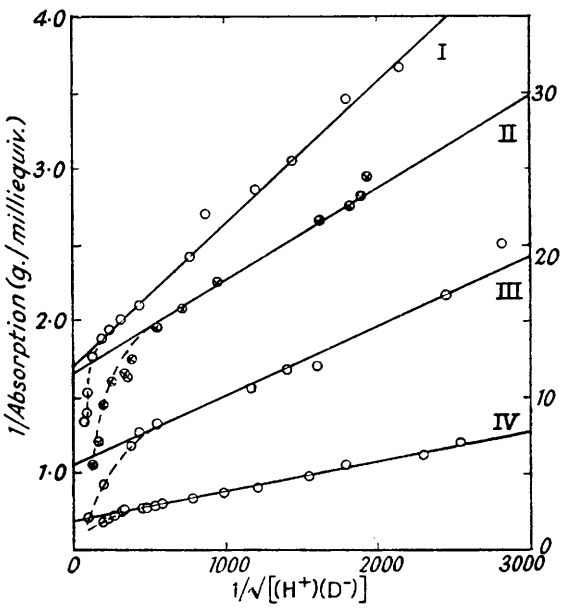


FIG. 4.



I, Standard polymer (a) & Orange II.
II, ,, ,, & Cardinal Red J.
III, Heated polymer (c) & Orange II (right-hand scale).
IV, Precipitated polymer (d) & Orange II.

The progressive decrease in the proportion of amino-groups available to dye in polyglycine I can be observed during the preparation of the polymer from piperazine-2 : 5-dione. Table 2 gives the milliequiv. of Orange II bound per g. of polymer prepared at 140°, with different periods of heating, and the subsequent change when the polymers were heated in air at 105—120°.

The results are not very reproducible, although there is a tendency for the dye-combining power to reach a constant value in water at 140°. But heating the polymer in air reduces the combining power continuously, though at a decreasing rate.

[7] Crick and Rich, *Nature*, 1955, **176**, 780.
[8] Astbury, Dalgleish, Damon, and Sutherland, *ibid.*, 1948, **162**, 596; Astbury, *ibid.*, 1949, **163**, 722.

TABLE 2. *Combination of polyglycine I with Orange II (polyglycine prepared from piperazine-2 : 5-dione at 140°; dione : water = 1 : 1)*

Reaction time (hr.)	19	24	42	114
Milliequiv. of orange II/g.	0·78	0·60	0·34	0·35
Heated at 105° in dry air.				
Days	1	3	5	
Milliequiv. of Orange II/g.	0·172	0·152	0·127	

The affinity of a monobasic acid for an insoluble polymer containing equal numbers of basic and acidic groups can be calculated from the following equation : [1c,9]

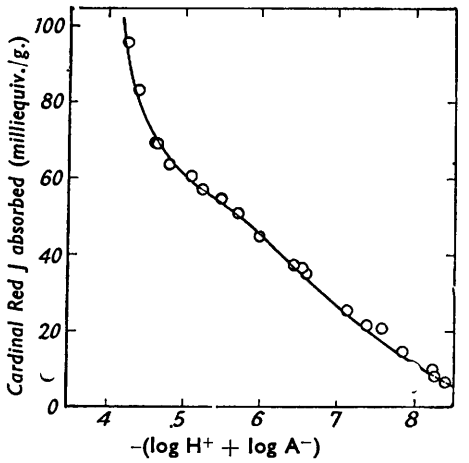
$$2\boldsymbol{R}T \ln \theta/(1-\theta) - \boldsymbol{R}T \ln (H^+) - \boldsymbol{R}T \ln (A^-) + \Delta\mu_{H^+} + \Delta\mu_{A^-} = 0 \quad . \quad (1)$$

where θ is the fraction of the total number of sites of the same charge which are occupied by ions of opposite charge, (H^+) and (A^-) are the activities of hydrogen ions and anions in the external aqueous phase. The plot of $\log \theta/(1-\theta)$ against $[\log (H^+) + \log (A^-)]$ should be a straight line with a slope of 0·5; and when $\theta = 0{\cdot}5$,

$$[\log (H^+) + \log (A^-)] = (\Delta\mu_{H^+} + \Delta\mu_{A^-})/2{\cdot}303\boldsymbol{R}T \quad . \quad . \quad . \quad (2)$$

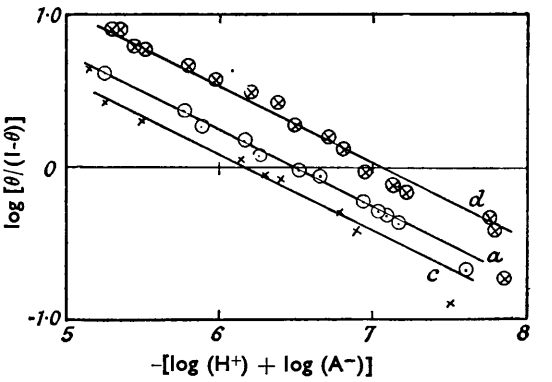
The plot of $[\log (H^+) + \log (A^-)]$ against $\log \theta/(1-\theta)$ is shown in Fig. 6 for Orange II and preparations (*a*), (*c*), and (*d*). In calculating θ, the available $-NH_2$ groups are taken as 0·60 milliequiv./g. for (*a*), 0·18 for (*c*), and 1·40 for (*d*). Although the points show

FIG. 5.



Standard polymer (a) and Cardinal Red J.

FIG. 6.



a, Standard polymer; c, heated polymer; d, precipitated polymer. All with Orange II.

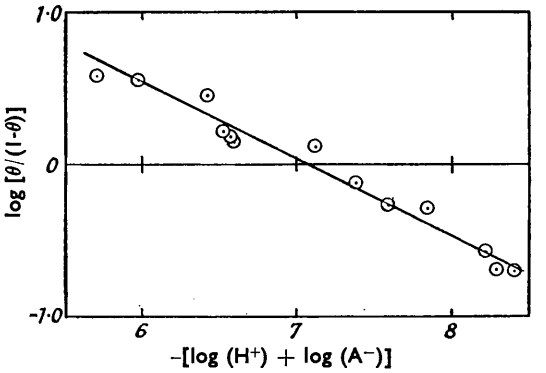


FIG. 7.
Standard polymer (a) & Cardinal Red J.

[9] Rideal and Gilbert, *Proc. Roy. Soc.*, 1943, *A*, **183**, 335.

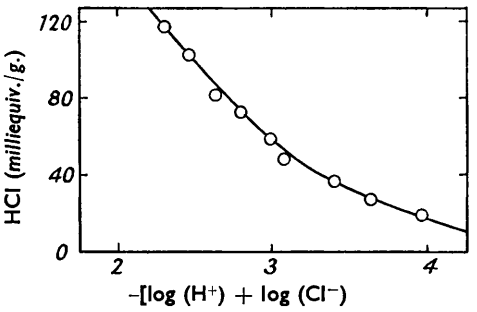
considerable scatter, they tend to lie along a line having the theoretical slope of 0·5 in all three cases. The value of $-$ [log (H^+) + log (A^-)] when $\theta = 0{\cdot}5$ is 6·5 for (*a*), 6·15 for (*c*), and 7·05 for (*d*) The corresponding values for the affinity, ($\Delta\mu_{H^+} + \Delta\mu_{A^-}$), are $-9{\cdot}9$, $-9{\cdot}35$, and $-10{\cdot}75$ kcal./mole.

A similar plot for the absorption of Cardinal Red J on preparation (*a*) is shown in Fig. 7. The value of $-$ [log (H^+) + log (A^-)] when $\theta = 0{\cdot}5$ is 7·07; ($\Delta\mu_{H^+} + \Delta\mu_{A^-}$) $= -10{\cdot}75$ cals.

The combination of hydrochloric acid with standard polymer (*a*) at 20° is shown in Fig. 8. Although the groups available to dye are 0·60 milliequiv./g. for this polymer, the amount of acid bound exceeds this figure, without any indication of an inflexion in the curve. Polymer washed in 0·1N-hydrochloric acid [(*b*), Table 1] contained 0·88 milliequiv. of acid per g. The reciprocal plot of Fig. 8 gives a curve passing through the origin. No indication of a maximum at 0·60 milliequiv./g. could be found. It seems that for polyglycine I absorption of hydrochloric acid on amide groups is a significant proportion of the total acid absorbed, even at very low acid concentrations.

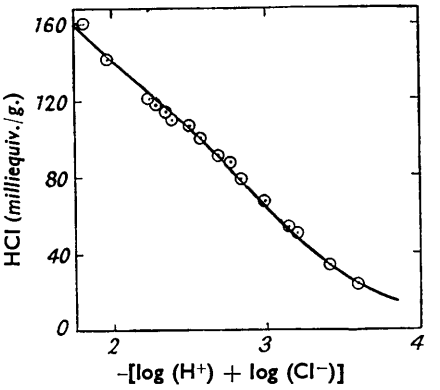
The corresponding curve for the precipitated polymer (*d*) at 20° is shown in Fig. 9. There is a slight indication of an inflexion at 1·00—1·20 milliequiv./g. However, the reciprocal plot passes through the origin, and no useful information can be obtained from it.

FIG. 8.



Standard polymer (a) and hydrochloric acid.

FIG. 9.



Precipitated polymer (d) and hydrochloric acid.

The solid line in Fig. 9 drawn through the experimental points was obtained in the following way: (1) Let it be assumed that $\Delta\mu_{Cl^-} = 0$; this is a hypothesis which has proved convenient, and appears to be approximately true for wool.[1a,c] (2) Hydrogen ions are absorbed on to the carboxyl groups of the polymer. The polymer contains 1·40 milliequiv. of CO_2^- per g., and the pK of the carboxyl group is 2·9. (3) Hydrogen ions are absorbed on to the amide groups of the polymer. The polymer contains 15·75 milliequiv. of CO·NH groups per g., corresponding to a degree of polymerization of 12·3, and the pK of the amide group is 0. The shape of the lower part of the curve is determined mainly by condition (2). 1·40 corresponds to the carboxyl content of the polymer, as determined by titration (Table 1). The value of 2·9 for the pK of the carboxyl group gives a good fit for the lower part of the curve, and agrees with the known values [5] for the higher peptides of glycine, which lie between 2·95 and 3·1. On assumptions (1) and (2), $\Delta\mu_H + \Delta\mu_{Cl} = \Delta\mu_H = -2{\cdot}303\boldsymbol{R}T \times 2{\cdot}9 = -3{\cdot}88$ kcal./mole at 20°.

The shape of the upper part of the curve is influenced to a considerable extent by the absorption of hydrogen ions on amide groups, the number of which is determined by the chemical composition of the polymer and its degree of polymerisation. In order to obtain a fit with the experimental values, it is necessary to assume that $K = 1$, *i.e.*, p$K = 0$, for the reaction, $\cdot CO\cdot NH_2^+\cdot \rightleftharpoons \cdot CO\cdot NH\cdot + H^+$. For acetamide p$K$ lies between $-0{\cdot}5$ and $-0{\cdot}9$ for this reaction, and for urea [10] between $-0{\cdot}05$ and $+0{\cdot}18$. The value postulated seems, therefore, to be reasonable, although no great significance should be attached to it.

[10] Hall and Conant, *J. Amer. Chem. Soc.*, 1927, **49**, 3047.

Discussion.—The absorption of the dye Naphthalene Scarlet 4R (1-naphthylamine-4-sulphonic acid→2-naphthol-6 : 8-disulphonic acid; C.I. No. 185) by polyglycine I has been studied by Bamford, Boulton, Hanby, and Ward.[11] A polyglycine having 0·164 milliequiv. of amino-groups per g. (by the Van Slyke method) absorbed 0·101 milliequiv. of the dye per g., *i.e.*, 62% of the theoretical amount. After refluxing with acetic anhydride for 15 min. the polymer contained 0·104 milliequiv. of NH_2 per g., but combined with only 0·024 milliequiv. of the dye, 23% of the theoretical amount. In a parallel experiment with nylon 6 : 6, dye absorption slightly exceeded the free amino-content, both before and after acetylation, presumably owing to absorption on amide groups under the conditions used. The difference in behaviour is the more striking in view of the similar crystal structure and chemical composition of the two substances.

These results are consistent with the observations on the absorption of Orange II by polyglycine I presented in this paper. Since heating at 105—110° decreases the number of amino-groups available to the dye, refluxing in acetic anhydride at 140° might also be expected to do so. But it is difficult to explain the effect, since polyglycine II, with the same degree of polymerization and the same chemical composition, and nylon 6 : 6, with a very similar crystal structure, do not show it. It might be argued that the undyed polymers are amorphous, and that heating causes crystallisation in polyglycine I, the amino-groups in the crystallites not being accessible to dye molecules. This however is not supported by *X*-ray diffraction. Polyglycine I, containing 0·76 milliequiv. of Orange II per g., equivalent to 25% on the weight of the undyed polymer, gave an *X*-ray diffraction pattern similar to that of the undyed material. Polyglycine II, containing 1·24 milliequiv. of Orange II (41% on the weight of the undyed material) also gave a diffraction pattern similar to that of the undyed material. It would be premature to say that dyeing produces no change in the *X*-ray diffraction pattern, but none has been found so far. It is known that the dyeing of textile fibres has no effect on the *X*-ray diffraction pattern (Speakman *et al.*[1]) and this has been used as evidence that dye absorption takes place in the amorphous regions of the fibres, but this cannot be reconciled with the behaviour of polyglycine II. Not only does the *X*-ray diffraction pattern show many sharp rings, indicating a high degree of crystallinity, but also certain preparations were found by electron-microscopy to consist of hexagonal laminæ, showing growth steps.[12] These were undyed preparations, but indications of a similar structure were found also in dyed preparations. Thus, there is evidence both from *X*-ray diffraction and from the electron micrographs that the material is crystalline; neverthless, it can absorb dye until nearly all available amino-groups are saturated, without loss of crystal structure and without marked change in the *X*-ray diffraction pattern.

Polyglycine II, the various types of nylon (Vickerstaff [1a]), wool,[1b] silk, and other insoluble proteins [2] combine with acid dyes in amounts corresponding to their content of basic groups. Only polyglycine I is an exception, for no obvious reason.

The affinity of Orange II for polyglycine II at 60° is −10·7 kcal./mole, from the results in Fig. 5.[1b,d,2] The affinity of the hydrogen ion for polyglycine II at 60° can be calculated from the results in Fig. 9, if it is assumed that pK does not change with temperature. This is equivalent to assuming that the heat of ionisation of the carboxyl group is zero, which is approximately correct.[5] Then $\Delta\mu_H = -4{\cdot}4$ kcal./mole at 60°, and for the absorption of Orange II anion by polyglycine II at 60°, $\Delta\mu_A = -(10{\cdot}7 - 4{\cdot}4) = -6{\cdot}3$ kcal./mole. This is a substantially greater affinity than that of the same anion on wool (−4·6 kcal./mole).

The affinity of Cardinal Red J is greater than that of Orange II on polyglycine I (standard polymer) by 0·85 kcal./mole, owing to the replacement of a benzene by a naphthalene nucleus and in agreement with results for wool. It is difficult to decide what significance to attach to the apparent difference in the affinity of Orange II for polyglycine I and polyglycine II, particularly as the affinity for standard polymer (*a*) differs from that of heated polymer (*c*), although both are polyglycine I. The number of groups accessible to dye differs in the two cases, and in calculating the affinities it has been assumed that the number of sites accessible to protons is equal to the number accessible to dye anions. If

[11] Bamford, Boulton, Hanby, and Ward, *Discuss. Faraday Soc.*, 1954, **16**, 222.
[12] Meggy and Sikorski, *Nature*, 1956, **177**, 326.

this assumption is not correct the affinities will also not be correct. It is quite possible that polyglycine I in which *all* potential sites are available to dye ions has the same affinity for Orange II as has polyglycine II.

Experimental

Preparation of the Polymer.—Piperazine-2 : 5-dione (10 g.) and water (10 g.) were heated in a sealed tube at 160—180° for 18 hr. The material from several tubes was bulked, extracted with boiling water three times, centrifuged, washed with methanol three times, and dried ($CaCl_2$) in a desiccator. The polymer (yield, 68%) was finely ground in a mortar, and stored over calcium chloride.

Determination of the Isoionic Point.—Polymer (0·5 g.) was weighed into each of several beakers containing distilled water (25 ml.) and 0·2N-sodium chloride (5 ml.). Suitable amounts of 0·1N-hydrochloric acid or -sodium hydroxide were added, and the pH of the solution in each beaker was measured after sufficient time for equilibration. N-Sodium chloride (5 ml.) was added and the pH of the solution at equilibrium measured again. The change in pH was plotted as a function of the initial pH. The isoionic point was found to be 5·1.

Some of the polymer was washed with an acetate buffer of pH 5·1. The polymer was washed with water and methanol, and dried ($CaCl_2$). The isoionic point was redetermined and found to be 5·1. The whole of the polymer was washed with acetate buffer (pH 5·1), then with water and methanol, and dried as described. This material is referred to as " stock polymer."

Preparation of Treated Polymers.—Precipitated polymer (*d*). Stock polymer (10 g.) was dissolved in neutral saturated calcium chloride solution (150 ml.). The solution was centrifuged and diluted with distilled water (600 ml.). The polymer which separated was washed at the centrifuge from distilled water four times, washed with acetate buffer of pH 5·1, then with water and methanol, and dried (yield 8·4 g., 84%).

Heated polymer (*c*). Stock polymer was heated in an air-oven at 105° for 10 days, during which no apparent change took place.

Heated and precipitated polymer (*e*). Heated polymer was precipitated from calcium chloride solution at room temperature and purified as for (*d*) above.

Precipitated and heated polymer (*f*). Polymer, precipitated as for (*d*), was heated for 10 days at 105° as for (*c*), washed with acetate buffer (pH 5·1), then with water and methanol, and dried ($CaCl_2$).

Titration of the Polymer in Lithium Bromide Solution.—Polymer (about 70 mg.) was dissolved in saturated lithium bromide solution (15 ml.), and titrated with freshly standardised N/15-sodium hydroxide after addition of 40% aqueous formaldehyde (1 ml.). A glass electrode was used to detect the end point, the reference electrode being a silver–silver bromide electrode in saturated lithium bromide solution, which was connected to the titration vessel by means of a wick soaked in the same solution. The E.M.F. of the system was measured with a Cambridge pH meter, used as a millivoltmeter, and as the E.M.F. was greater than 1400 mv a standard cell was included in the circuit to bring the readings within the scale of the instrument. A blank titration was carried out to allow for the acidity of the formaldehyde; the lithium bromide solution was neutral.

Combination of the Polymer with Dye Acids.—(1) *Purification of the dye acids.* Technical Orange II sodium salt (Imperial Chemical Industries Limited, Dyestuffs Division; Naphthalene Orange G) was crystallised twice from warm water, then dissolved in cold water (200 ml. for 18 g.) and treated with concentrated hydrochloric acid (10 ml.). The precipitated dye acid was separated at the centrifuge, redissolved, and again precipitated with acid. The precipitate was washed three times with 0·5N-acid and dried (NaOH). It was then recrystallised twice from absolute alcohol, washed with a little ether, dried (NaOH), and stored over concentrated sulphuric acid in the dark.

Technical Cardinal Red sodium salt (Imperial Chemical Industries Limited; Naphthalene Red JS) was recrystallised twice from water at 60°. The crystals must be separated at the centrifuge. Conversion into the dye acid and purification were as for Orange II.

In both cases the dye acids gave a negative reaction for chloride with silver nitrate. For Orange II and Cardinal Red respectively the ash content was 0·3% and 0·2% and the purity by potentiometric titration with alkali was 101·1% and 100·6%.

(2) *Estimation of the dyes in solution.* The dyes were estimated with a Spekker absorptiometer, and Ilford Spectrum filters, No. 603, blue-green for Orange II, and No. 604, green for

Cardinal Red. For concentrations of dye in solution less than 4×10^{-5}M, the Spekker reading was proportional to the concentration; aliquot parts of solutions to be measured were diluted to bring them within the range $1—4 \times 10^{-5}$M.

(3) *Equilibrium measurements.* Weighed amounts of polymer and known volumes of 0·01N-dye solution were placed in tubes fitted with Quickfit stirrers, to prevent loss of water by evaporation. The tubes were immersed in a thermostat at 60° and stirred. Preliminary experiments showed that equilibrium was attained after 6 hr. The tubes were cooled rapidly to room temperature and the contents centrifuged. The centrifuge tubes were weighed before and after centrifugation, and any loss of water by evaporation was made good. A portion of the clear liquid was used in determination of the pH of the system, and a second portion in determination of the residual dye concentration. From the volume of dye solution taken and the initial and the final concentration, the amount of dye on the polymer was calculated.

Combination of the Polymer with Hydrochloric Acid.—Polymer (about 1 g.) was weighed into a stoppered flask (50 ml.), and a known volume of standardised hydrochloric acid added. The flask was rotated for 6 hr. (sufficient for equilibration). A portion of the liquid was centrifuged at high speed to separate the polymer, which sometimes tended to remain in suspension, and any loss by evaporation was made good. An aliquot part was titrated with barium hydroxide so ution, giving the amount of acid bound.

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