683. *Chemical Actions of Ionising Radiations on Aqueous Solutions. Part IV. The Action of X-Rays on Some Amino-acids.*

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The action of *X*-rays on glycine, alanine, and serine in aqueous solutions has been studied under different conditions, *e.g.*, in the presence of oxygen (air), in a vacuum, in hydrogen, at different pH values, at different concentrations, etc. It has been found that a deamination occurs and that ammonia and other products are formed. From the latter the corresponding aldehydes have been isolated as their 2 : 4-dinitrophenylhydrazones. The amounts of the ammonia, aldehydes, and molecular hydrogen formed in the reaction were shown to be a non-linear function of the dose.

A number of features of the mechanism of deamination have been discussed and we have concluded that, in general, an oxidative and a reductive mechanism are operative in the deamination of amino-acids. In particular, when *X*-rays act on aqueous solutions, producing hydroxyl radicals and hydrogen atoms, both radicals are presumably capable of initiating deamination.

The results already reported (Parts I—III; preceding papers) of our investigations on the action of ionising radiation on aqueous solutions are in agreement with the free-radical theory of indirect action (Weiss, *Nature*, 1944, **153**, 748; *Trans. Faraday Soc.*, 1947, **43**, 314). The qualitative and quantitative results point to the formation of hydrogen atoms and hydroxyl radicals from the water, following the absorption of the radiation. The present investigation had as its object to obtain some information regarding the action on amino-acids in aqueous solutions, and at the same time it was to serve as a first step in the investigation of more complex molecules which are of biological significance.

The action of hydroxyl radicals, produced by means of Fenton's reagent (hydrogen peroxide–ferrous salt), on simple amino-acids (glycine, alanine, serine, etc.) has been investigated qualitatively in the past (Dakin, *J. Biol. Chem.*, 1906, **1**, 171; Neuberg, *Biochem. Z.*, 1909, **20**, 534). An oxidative deamination occurs, yielding ammonia, an aldehyde and carbon dioxide (which results from a subsequent decarboxylation), whilst in biological oxidative deaminations the corresponding keto-acid is sometimes formed. These results obtained with Fenton's reagent are similar to those of electrolytic oxidation (Neuberg, *ibid.*, 1910, **24**, 159; Fichter, *Helv. Chim. Acta*, 1920, **3**, 712; Baur, *Z. Elektrochem.*, 1936, **42**, 285), which presumably also involves free-radical intermediates (cf. Walker and Weiss, *Trans. Faraday Soc.*, 1935, **31**, 1011). Weizmann, Bergmann, and Hirschberg (*J. Amer. Chem. Soc.*, 1936, **58**, 1675) have investigated the photochemical (hydrolytic) deamination of some amino-acids. This does not necessarily proceed by a mechanism similar to that which operates with ionising radiations, since in the photochemical experiments the primary energy absorption is due entirely to the amino-acid. Loiseleur (*Compt. rend. Soc. Biol.*, 1933, **114**, 589) has demonstrated qualitatively the deamination of amino-acids by α-particles. In this case, however, hydrogen peroxide is presumably formed (see, *e.g.*, Part III). No other products were identified by him.

Should the action of *X*-rays (in the absence of oxygen) give results similar to those obtained

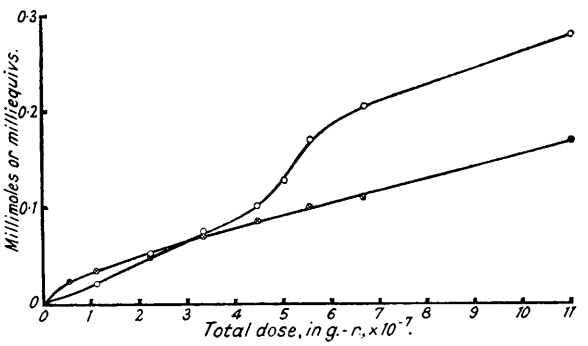
by the action of hydroxyl radicals produced by means of the hydrogen peroxide–ferrous salt system (Haber and Weiss, *Proc. Roy. Soc., A*, 1934, **147**, 332), the theory of the free-radical mechanism of the indirect mode of action of ionising radiations would receive further support. The fact that deamination occurs could be of some significance from the biological point of view since Auerbach (*Proc. Roy. Soc. Edinburgh, B*, 1947, **62**, 284) has shown that ammonia is capable of inducing mutations in *Drosophila*. Since amino-acids are initimate constituents of the living cell, the production of ammonia *in situ* might have significant effects.

Qualitative Experiments.—Glycine, alanine, and serine were investigated qualitatively. The arrangements for the irradiations were those described in detail in Part I (*loc. cit.*). In all cases 400 mg. of the amino-acid in 100 ml. of water were irradiated, in a vacuum, with doses of the order of 10^5—10^6 r. Hydrogen and ammonia were given off by all these amino-acids.

Glycine yielded very small quantities of formaldehyde, alanine very small quantities of acetaldehyde, and serine relatively larger quantities of glycollaldehyde and glyoxal. All these aldehydes were isolated as the 2 : 4-dinitrophenylhydrazones. Fricke's work (see, *e.g.*, Fricke, Hart, and Smith, *J. Chem. Physics*, 1938, **6**, 229) has shown that formaldehyde and acetaldehyde, even in very dilute solutions are decomposed under the influence of *X*-rays. The action of *X*-rays on glycollaldehyde has not been investigated previously. It seems likely that the glyoxal is the result of the action of *X*-rays on the primarily formed glycollaldehyde. Keto-acids could not be isolated in any of these experiments. Full details are given in the Experimental section.

FIG. 1.

Irradiations of aqueous solutions of glycine (400 *mg.*/100 *ml.*) *in a vacuum.*



Yields of hydrogen equiv. (○) *and of ammonia* (⊗) *plotted against total dose of* X-*rays.*

Our preliminary results on amino-acids have been briefly published (Stein and Weiss, *Nature*, 1948, **162**, 814). Dale and Davies (*ibid.*, 1949, **163**, 64) subsequently confirmed our results regarding the deamination and drew attention to the fact that the yield of ammonia shows an unexpectedly strong dependence on the initial concentration of glycine. When relatively high concentrations of glycine (of the order of 10—20 g./100 ml.) were used the yield of ammonia was nearly three times as high as was expected on the basis of one molecule of ammonia formed for one ion pair. (Dr. Dale has pointed out to us that this effect is not influenced by the presence of oxygen.) However, these authors did not report or investigate any other products of the reaction.

Quantitative Experiments.—In Figs. 1, 2, and 3 the yields of ammonia and of hydrogen equivalents are plotted against the total dose, for glycine, alanine, and serine, respectively. All these experiments were carried out in a vacuum, and in all of them the concentration of amino-acid was 400 mg./100 ml. of solution. (The pH of the solutions was not specially adjusted.) At these relatively low concentrations direct-hit effects can be neglected. The three curves show the same trend, shown most clearly by glycine (Fig. 1) which was investigated in greater detail. The yield of ammonia *per unit dose* is at first high (see also Fig. 5), but decreases gradually to a lower, practically constant value. The yield of hydrogen is represented in the initial stages by a sigmoid curve.

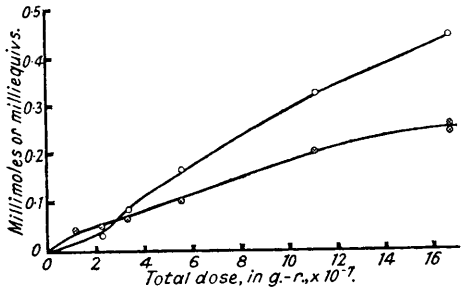
Although the yields of phenol and hydrogen obtained from benzene (Part II, *loc. cit.*) were constant at low doses, in this case the yields of ammonia and hydrogen change continually, and the relative yield at a given total dose is a function of that particular dose. Thus both the experiments of Dale and Davies (*loc. cit.*, medium doses of the order of 10^5 r.) and our earlier

qualitative experiments (high doses of the order of 10^6 r.) refer to particular doses only. From the Figures 1, 2, 3, and 5 it is seen that as the total dose decreases the yield of ammonia progressively increases. Thus, the ammonia-yield curves obtained suggest that one is dealing here with a competition by at least two acceptors for the available radicals, one of the acceptors being the amino-acid and the second, a product of the primary reaction. This second product accumulates slowly and even when present in small amounts appears to be capable of reducing the attack on the amino-acid. However, as a result of these two processes a practically constant rate of production of ammonia (from the amino-acid) is eventually reached (cf. Figs. 1 and 3).

The hydrogen-yield curves are also compatible with this view, *i.e.*, of two reactants competing for the radicals. It would appear that the hydrogen is produced as the result of two processes. For instance, if the primary attack on the amino-acid yields ammonia and a second product (*e.g.*, an aldehyde or an acid) which is itself capable of further decomposition by the radicals with the formation of hydrogen, then as the result of the competing reactions a constant rate of production of hydrogen will be reached when the concentration of the intermediate product has attained a stationary (equilibrium) level. Up to this point, however, the scheme of two consecutive reactions can result in a sigmoid curve representing the yield of hydrogen against dose (Fig. 1).

FIG. 2.

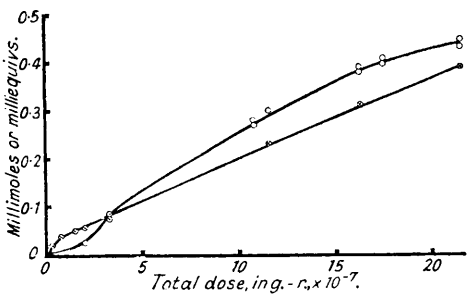
Irradiations of aqueous solutions of alanine (400 mg./100 ml.) in a vacuum.



Yields of hydrogen equiv. (○) and of ammonia (⊗) plotted against total dose of X-rays.

FIG. 3.

Irradiations of aqueous solutions of serine (400 mg./100 ml.) in a vacuum.



Yields of hydrogen equiv. (○) and of ammonia (⊗) plotted against total dose of X-rays.

This can be seen easily if one considers the transformation A ⟶ B by means of the active radicals R, by the consecutive reactions :

$$A + R \xrightarrow{k_\alpha} X \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (\alpha)$$

$$X + R \xrightarrow{k_\beta} B \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (\beta)$$

where A denotes the original acceptor (amino-acid), X is the intermediate competing with A for the active radicals (R), and B corresponds to the hydrogen formed by the further decomposition of X.

Under conditions of irradiation with a constant dose rate the concentration of the active radicals can be considered as practically constant. In this case, the initial stages of the reaction, *i.e.*, before the intermediate product (X) has reached a stationary state (for which $dX/dt = 0$), equations (α) and (β) lead to an expression for the end product (B) which is a function of the time :

$$[B]_t = [A]_0\left\{1 + \frac{1}{(k_\beta - k_\alpha)}\left[k_\alpha e^{-k_\beta[R]t} - k_\beta e^{-k_\alpha[R]t}\right]\right\}$$

This represents a sigmoid curve of the type discussed above. The total dose is proportional to the time, for a given constant dose rate [t = (total dose)/(dose rate)]. From the above equation one obtains for the point of inflexion the expression :

$$t_{\text{infl.}} = \frac{1}{(k_\beta - k_\alpha)[R]} \ln \frac{k_\beta}{k_\alpha}$$

which gives a positive value only if $k_\beta > k_\alpha$, *i.e.*, if the rate constant of the second stage (equation β) is greater than that of the first stage (equation α).

Fricke *et al.* (*loc. cit.*) have shown that aqueous solutions of formaldehyde, formic acid, acetaldehyde, and acetic acid give hydrogen when irradiated by *X*-rays. Geib (*Ergebn. exakt.*

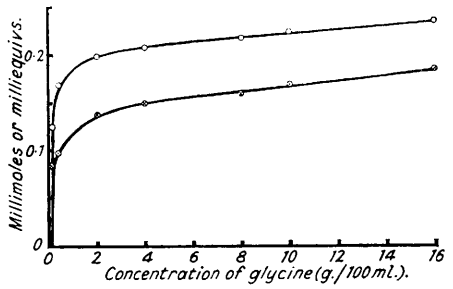
Naturwiss, 1936, **15**, 44) has summarised the evidence that hydrogen atoms easily decompose these substances.

Such a competition would also account for the results of Dale and Davies (*loc. cit.*) regarding the influence of the initial concentration on the yield of ammonia and is confirmed by our experiments. Fig. 4 shows the yields of ammonia and hydrogen obtained by us from glycine at a constant dose of 150 minutes (approx. 5×10^7 g.-r.) in an unbuffered solution, at concentrations of glycine up to 16 g./100 ml. of solution. At such high concentrations the energy absorbed cannot be assumed to be the same as that in the case of dilute solutions, since the absorption of the radiation depends on the density of the solution and on the average atomic number of the medium. Calculations of these effects, carried out by Mr. M. J. Day, have shown that for glycine the effect of increased density (1·04 for 10 g./100 ml. of solution) is approximately counteracted by the effect of the lower average atomic number of glycine compared with that of water. To a first approximation, therefore, the dose in concentrated solutions of glycine is the same as in dilute solutions. Direct hits are however more frequent in the concentrated solutions.

Fig. 4 shows that the yield of ammonia increases slightly up to the highest concentrations investigated, whilst the yield of hydrogen does not increase markedly after a maximum value

FIG. 4.

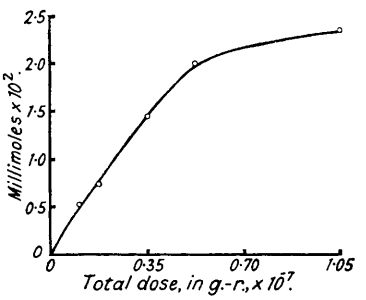
Irradiations of aqueous solutions of glycine in a vacuum.



Dependence of the yields of hydrogen equiv. (○) and ammonia (⊗) on the concentration of glycine, at a constant dose of approx. 5×10^7 g.-r.

FIG. 5.

Irradiations of aqueous solutions of glycine (400 mg./100 ml.) in a vacuum at low doses.



Yields of ammonia plotted against total dose of X-rays.

has been reached at medium concentrations. This can be attributed presumably to two causes: (i) a mechanism involving competing reactions as discussed above, and (ii) the possibility that at these higher concentrations direct hits might result in a hydrolytic reaction such as that described by Weizmann *et al.* (*loc. cit.*) for the photochemical reaction which would give some ammonia but not hydrogen.

The shape of the ammonia-yield curve in Fig. 4 is in agreement with the equation derived by Weiss (cf. *Nature*, 1944, **153**, 748; Part I, *loc. cit.*) for reactions in which competition occurs.

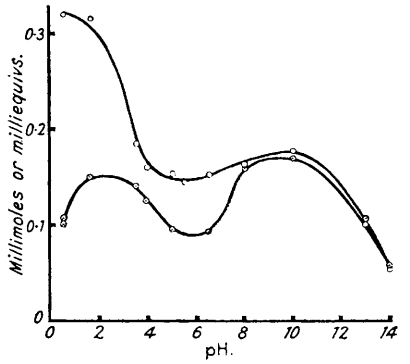
As mentioned above, the relatively higher yields of ammonia become more pronounced with decreasing total dose. To study the initial conditions we measured (Fig. 5) the yield of ammonia obtained at very low doses in a vacuum from an unbuffered solution of glycine (400 mg./100 ml.), *i.e.*, under the conditions of the experiments of Fig. 1. Already after 15 minutes (approx. dose 5×10^4 r.) the rate of formation of ammonia decreased significantly. The initial yield, calculated in terms of an assumed energy absorption of 32·5 ev. per ion pair formed, amounts to $M/N = 1{\cdot}85$.

The dependence of the yields of ammonia and hydrogen (in a vacuum) on variations in pH is shown in Figs. 6 and 7. Fig. 6 shows the yields of ammonia and equivalents of hydrogen at a constant dose of 150 minutes of irradiation (approx. 5×10^5 r.) and for a constant concentration of glycine of 400 mg./100 ml. of solution. At this higher dose the analytical accuracy and reproducibility is very good. However, owing to the secondary reactions mentioned above the yields obtained at this dosage are the result of several processes. Fig. 7 was obtained for the same concentration of glycine, but at a constant dose of one-fifth of the previous one, *i.e.*, of 30 minutes (approx. 1×10^5 r.). In this case, therefore, the secondary reactions play a smaller role. The general trend of the curves in both figures is similar. The yield of hydrogen, as the

result of the secondary reaction discussed above, exceeds that of ammonia at the higher dose at acid, but not at alkaline, pH values. A similar influence of pH on the yield of hydrogen (owing to the attack on the reaction products, aldehydes etc.) has been found also by Fricke *et al.* (*loc. cit.*) using formaldehyde.

FIG. 6.

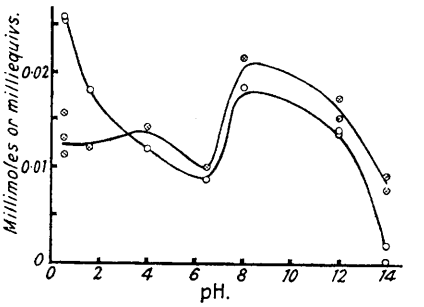
Irradiations of aqueous solutions of glycine (400 mg./100 ml.) in a vacuum.



Dependence of the yield of hydrogen equiv. (○) and of ammonia (⊕) on pH at a constant dose of approx. 5 × 10⁵ r.

FIG. 7.

Irradiations of aqueous solutions of glycine (400 mg./100 ml.) in a vacuum.



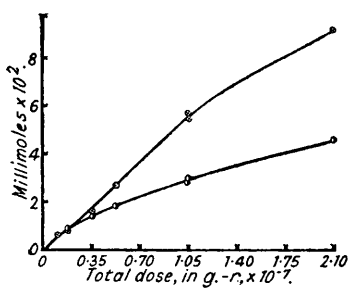
Dependence of the yield of hydrogen equiv. (○) and of ammonia (⊗) on pH at a constant dose of approx. 1 × 10⁵ r.

The yield of ammonia depends on the pH in a very similar way in both cases (Figs. 6 and 7). Starting at the lowest pH values, the yield rises to a maximum at pH ~3, falls to a pronounced minimum at a pH ~6—6·5, rises very steeply to a second maximum around pH ~9, and falls again at higher pH values. Of the two maxima, that at pH ~9 is the greater.

In agreement with Dale and Davies (*loc. cit.*) it was found that the yield of ammonia remained the same whether experiments were carried out in the presence of air or in a vacuum. We found that it was also unchanged in an atmosphere of hydrogen.

FIG. 8.

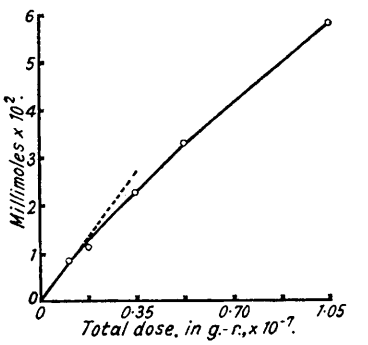
Irradiations of aqueous solutions of glycine (2 g./100 ml.) at pH 8, i.e., under conditions giving approximately the maximum initial yield.



Yields of ammonia (⊗) and of aldehyde (◑) plotted against total dose of X-rays.

FIG. 9.

Irradiations of aqueous solutions of serine (400 mg./100 ml.) at pH ~8 in hydrogen.



Yields of ammonia plotted against total dose at a constant dose rate of approx. 3500 r. per min.

The yield of aldehyde, however, depended on these factors. From glycine we obtained the highest yield in air, a somewhat lower yield in a vacuum, and the lowest in hydrogen.

This can be interpreted on the basis that, in the case of glycine, *not only the hydroxyl radicals, but also the hydrogen atoms attack the acceptor, both leading to the formation of ammonia.* The other product is apparently an aldehyde when the attack is by hydroxyl (or by HO_2, in the presence of oxygen), but is not an aldehyde when the attack is by hydrogen atoms. This view, based on

the above-mentioned facts, is supported by other evidence discussed below. If this is so, a value of $M/N \approx 2$ would be expected for the yield of ammonia obtained in the initial stages of the reaction.

Since the yield of ammonia had been found to depend, not only on the concentration of the amino-acid and on the total dose given, but also on the pH, experiments were next made under conditions giving approximately the maximum initial yield. Fig. 8 gives the yields of ammonia and aldehyde obtained in a buffered solution at a concentration of 2 g. of glycine per 100 ml. and in the presence of oxygen (air). The pH chosen (~ 8) corresponds approximately to the conditions of maximum yield; the yield at the concentration chosen approaches (cf. Fig. 4) the maximum reasonably closely without the likelihood of direct hit effects influencing the result; and the presence of air increases the yield of aldehyde without influencing the yield of ammonia (cf. above). The M/N value obtained initially for ammonia was 2·3. Comparison of Figs. 8 and 5 shows that the decrease in the rate of formation of ammonia sets in later under the conditions represented by Fig. 8, and that the *rate of formation* of the aldehyde in the solution declines fairly rapidly.

If the explanation that both hydroxyl radicals and hydrogen atoms lead to deamination is correct, the finding that the presence of absence of oxygen does not influence the yield of ammonia must be due to the fact that OH, HO_2, and H react with about the same efficiency in the case of glycine.

That this is not always the case was shown by experiments with serine. In this case, the yield of ammonia was found to be the lowest in the presence of air, higher in a vacuum, and highest in an atmosphere of hydrogen (all measurements at a dose of approx. 1×10^5 r.). In air the dialdehyde is formed, whilst in a vacuum or in hydrogen the monoaldehyde predominates. This indicates, that in the case of serine, the second product (*i.e.*, the monoaldehyde), once formed, is itself easily attacked by the radicals produced in the presence of oxygen, but that in hydrogen the hydrogen atoms (which predominate; see Part II) lead to a preferential deamination.

Fig. 9 records an attempt to determine the *initial yield* from serine, the concentration being 400 mg./100 ml. at pH 8, in hydrogen. The initial yield gives a value of $M/N = 2{\cdot}8$ for ammonia.

The results obtained indicate, that for amino-acids, the marked dependence of the yield of ammonia on the concentration of the acceptor is due to a competition for the radicals by the secondary products formed in the initial deamination reaction. This view is supported independently by the study of the yield–dose curves.

The very high yields of ammonia observed under certain conditions are presumably due to several reasons. The nature of the reactions involved leads one to expect much higher yields than were observed from, for example, benzene. Thus it was shown that the formation of phenol was a two-step process: the initial attack by the radicals led to the formation of phenyl radicals, and the second reaction, the formation of phenol, had to compete with a number of other reactions of these radicals; had we been able to measure the total *primary* formation of phenyl radicals much higher "yields" of these would probably have been observed. On the other hand, with amino-acids the very first attack by the radicals leads apparently to deamination; and, if only one type of radical is involved, a yield of ammonia approaching unity would be expected.

The hypothesis that both hydroxyl radicals and hydrogen atoms can deaminate the acid is strongly supported by our experimental results. Other evidence also supports the view that hydrogen atoms are capable of deaminating amino-acids. Thus Baur (*loc. cit.*) showed that amino-acids are deaminated electrolytically at the cathode. Kocholaty and Hoogerheide (*Biochem. J.*, 1938, **32**, 437), using anaërobic bacteria in the presence of molecular hydrogen, observed the reductive deamination of glycine, coupled with the uptake of hydrogen, and the formation of ammonia and of a (saturated) fatty acid.

That hydrogen atoms are formed in the irradiation of aqueous solutions has been further demonstrated by our experiments (unpublished), where solutions of gold, silver, and mercury salts were irradiated with *X*-rays. Whilst in the presence of the oxygen (air) very little or no reduction occurred, there was appreciable reduction in a vacuum which became much greater when the irradiated solution was in an atmosphere of hydrogen. The reactions which are to be taken into account in the presence of these gases have been discussed in Part II.

If with amino-acids in solution deamination by hydroxyl radicals and hydrogen atoms does take place, yields of up to *twice* the accepted *maximum value* are to be expected.

The observed dependence of the yield on pH might be connected with the state of the amino-acids in solution (cf. Edsall, *e.g.*, in Cohn and Edsall, "Proteins, Amino-acids, and Peptides,"

1943). The zwitterion form of, *e.g.*, glycine ($^{+}NH_3 \cdot CH_2 \cdot CO_2^{-}$), which predominates in aqueous solutions at the isoelectric point is in equilibrium with the cation ($^{+}NH_3 \cdot CH_2 \cdot CO_2H$) at acid, and with the anion ($NH_2 \cdot CH_2 \cdot CO_2^{-}$) at alkaline, pH values. The isoelectric point of glycine is at pH 5·97; around this pH value the zwitterion structure predominates. It is around this pH value that the pronounced minimum in the yield of ammonia was observed. There are also two other points of equilibria in aqueous solutions of glycine, *viz.*, where the concentration of the zwitterions equals that of the cations (on the acid side) and where the concentration of zwitterions and anions are equal (on the alkaline side). For glycine these equilibria are at pH values of 2·35 and 9·8 respectively, approximately coinciding with the regions of the two maxima in the yield of ammonia.

In microbiological work with amino-acids (see, *e.g.*, Stephenson, " Bacterial Metabolism," 1949) the so-called Stickland reaction (mutual oxidation and reduction by pairs of amino-acids, resulting in the deamination of both amino-acids) has been observed. The amino-acids can be divided into oxidisable (alanine, valine, etc.) and reducible acids (glycine proline, etc.). In general, an amino-acid of either group, when incubated alone, gives little or no ammonia; if an amino-acid of the other group is added strong deamination of both occurs. Recently, it has been shown (Cardon and Barker, *Arch. Biochem.*, 1947, **12**, 165) that the same reaction can occur in solutions containing only one amino-acid, two molecules of the same acid entering into this type of mutase reaction. It is not impossible that if such a reaction takes place the optimum conditions for it will be those under which different ionic structures of the same amino-acid are present, one structure being reduced while the other is oxidised. The same could favour the formation of a dimeric form of the amino-acid.

The results of Dale and Davies (*loc. cit.*) regarding yields of ammonia of $M/N > 2$ are thus confirmed. We are able partly to account for it, although we cannot at the moment go much beyond the points discussed above. In the absence of any definite knowledge regarding the primary ionic (radical) yields in solutions it is impossible to say whether the results hitherto obtained do in fact already exceed the real primary yield by a factor greater than two.

It is also quite possible, that in the process of deamination, reactive fragments (radicals) of the amino-acid are produced, which can then react with another amino-acid molecule, resulting in further deamination (short chains).

In any case it is indicated by the strong pH effects that in the case of acceptors giving ions in solution ionic equilibria might be of great importance.

Experimental.

Glycine (" AnalaR "), alanine (Hopkin and Williams), and serine (Roche) were used without further purification. The control ammonia values were determined for every new batch and allowed for.

The arrangements for irradiation were those described in Part I (*loc. cit.*). The determination of hydrogen was carried out as described in Part II (*loc. cit.*), the analytical procedure being accurate to $\pm$ 1% with 0·1 millimole of gas. Fricke (*loc. cit.*) has shown that even the most carefully distilled water gives off some hydrogen during irradiation. In the presence of other acceptors dissolved in the water, the hydrogen from this source is probably negligible; our double-distilled water (which was always used) gave when irradiated in the absence of any dissolved substances somewhat less than 10^{-6} mole per 10^{7} g.-r.

Determination of Ammonia.—Irradiated solutions were steam-distilled in a vacuum according to Parnas (*Biochem. Z.*, 1934, **274**, 158; Krebs, *Biochem. J.*, 1935, **29**, 1620), and the ammonia determined in the distillate by Nesslerisation and at larger doses also by titration.

The quantitative colorimetric micro-determinations were carried out with the Nesslerised solution in a Spekker colorimeter with an Ilford filter 601 or 602. The calibration was made with recrystallised " AnalaR " ammonium chloride.

Qualitative Separation of Aldehydes.—The irradiated solutions were slightly acidified with dilute sulphuric acid, and a solution containing 2·5 mg. of 2 : 4-dinitrophenylhydrazine per ml. of 2N-sulphuric acid was added. The precipitate was filtered off, washed, dried, and purified by chromatography on alumina. Glycine gave the formaldehyde, and alanine the acetaldehyde, derivative identified by their m. p.s (not depressed by admixture with authentic specimens). Serine gave a larger amount of precipitate which on chromatography was separated into two fractions. The first hydrazone formed orange crystals, m. p. 156°, not depressed by admixture with glycollaldehyde dinitrophenylhydrazone, which we prepared according to the procedure of Collatz and Neuberg (*Biochem. Z.*, 1932, **255**, 27). The second fraction formed dark red plates, m. p. 315—320°; the m. p. of glyoxal dinitrophenylhydrazone is 327°; our sample gave a violet colour, characteristic of dialdehyde derivatives, in alkaline solution.

Quantitative Determination of the Aldehydes.—To determine concentrations of aldehydes of the order of 10^{-5} mol./100 ml., the following procedure has been adopted. Dinitrophenylhydrazones of aldehydes, ketones, and keto-acids dissolve in alkalis with a bright cherry-red colour; the derivatives of dialdehydes give a violet colour. Bamberger (*Ber.*, 1893, **26**, 1306) used the *p*-nitrophenylhydrazones; Dakin (*J. Biol. Chem.*, 1908, **4**, 235) used this reaction for the detection of pyruvic acid; Friedemann and Haugen (*J. Biol. Chem.*, 1943, **147**, 415) adapted it for the quantitative micro-determination of ketonic substances in blood and urine, using the colour developed when the hydrazone is extracted into an

organic solvent; in this case the colour is measured in a volatile solvent and is not nearly as intense as the colour developed when the organic solution is shaken with alkali; the colour is however unstable in alkali. We found that, at room temperature, this colour in alkali fades to a definite value of yellow in about 12 hours, remaining then stable for long periods. Warming the solution accelerates this process, and constant values are obtained within reasonable times.

Our procedure, which permits determination of aldehyde at concentrations of the order of 10^{-5} mole/100 ml., consists of withdrawing an aliquot of the irradiated solution, neutralising it, adding 1 ml. of N-sulphuric acid, and making up the volume with water to exactly 20 ml. Exactly 5 ml. of the dinitrophenylhydrazone solution (see above) are added, and after 10 minutes the solution is extracted with 25 ml. of carbon tetrachloride, which as found by Lester and Greenberg (*J. Biol. Chem.*, 1948, **174**, 903) dissolves very little of the unchanged reagent and relatively large amounts of the hydrazone. The carbon tetrachloride extract is separated, shaken with 10 ml. of 2N-sulphuric acid and then with 10 ml. of 2N-sodium hydroxide, and the alkaline solution, in a 50-ml. graduated flask, is diluted to approx. 30 ml., heated in a boiling water-bath for 5 minutes, cooled, and diluted to volume. The colour is measured in a Spekker colorimeter, with an Ilford No. 601 filter, calibrated with "AnalaR" formaldehyde and acetaldehyde, or, for glycollaldehyde, with the purified dinitrophenylhydrazone prepared as described above. Results are reproducible within $\pm 3\%$ when the amount of formaldehyde is of the order of 1×10^{-5} mole in 50 ml. of the final (alkaline) solution.

The irradiations were carried out in the Radium Department of the Royal Victoria Infirmary. We are indebted to Mr. J. Thurgar, Dr. F. T. Farmer, and Mr. M. J. Day for their co-operation. This work was supported by grants from the Northern Council of the British Empire Cancer Campaign to whom our grateful thanks are due.

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[*Received, August 3rd*, 1949.]