siderations and the nature of the end products; all the ammonia was liberated from the hexosamines, whereas two amino groups would remain fixed in ornithine if arginine were an intermediate. Nevertheless, the extremely poor array of deaminases present in the streptococci renders desirable a further examination of the relations between arginine dihydrolase and the deaminase active in the breakdown of the amino sugars.

SUMMARY

1. The rates at which suspensions of *Streptococcus haemolyticus* and *Strep. viridans* utilize *N*-acetylglucosamine show no obvious correlation with the amount of hexosamine synthesized by cultures.

2. The end products formed by streptococci from 1 mol. of *N*-acetylglucosamine are 1 mol. of ammonia, 1.2–1.7 mol. of lactate and 1 equivalent of volatile acid.

3. The pH optimum for the reaction is 7.2–7.5.

4. The rates of utilization, deamination, and lactate formation are equal.

5. Reagents inhibiting lactate formation also inhibit deamination and the disappearance of *N*-acetylglucosamine.

6. The most likely mechanism for the reaction appears to be the breakdown of the glucopyranose structure with the subsequent deamination of an intermediate amino compound.

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REFERENCES

- Clark, W. M. (1928). *The Determination of Hydrogen Ions*, 3rd ed. London: Baillière, Tindall and Cox.
- Conway, E. J. (1947). *Micro-diffusion Analysis and Volumetric Error*. London: Lockwood and Sons Ltd.
- Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
- Friedemann, T. E., Cotonio, M. & Shaffer, P. A. (1927). *J. biol. Chem.* **73**, 335.
- Hewitt, L. F. (1932). *Biochem. J.* **26**, 208.
- Hills, G. M. (1940). *Biochem. J.* **34**, 1057.
- Kendall, F. E., Heidelberger, M. & Dawson, M. H. (1937). *J. biol. Chem.* **118**, 61.
- Lutwak-Mann, C. (1941). *Biochem. J.* **35**, 610.
- McClean, D. (1941). *J. Path. Bact.* **53**, 13.
- McIlwain, H. (1946). *Biochem. J.* **40**, 67.
- Michaelis, L. (1930). *J. biol. Chem.* **87**, 33.
- Morgan, W. T. J. (1936). *Biochem. J.* **30**, 909.
- Morgan, W. T. J. & Elson, L. A. (1934). *Biochem. J.* **28**, 988.
- Rogers, H. J. (1945). *Biochem. J.* **39**, 435.
- Rogers, H. J. (1946). *Biochem. J.* **40**, 782.
- Rogers, H. J. (1948). *Nature, Lond.*, **161**, 815.
- Rosenberg, A. J. (1948). *C.R. Acad. Sci., Paris*, **226**, 1751.
- Smith, P. A. & Sherman, J. M. (1941). *J. Bact.* **41**, 101.
- Somogyi, M. (1937). *J. biol. Chem.* **117**, 771.
- White, T. (1940). *J. Chem. Soc.* p. 428.
- Winnick, T. (1942). *J. biol. Chem.* **142**, 451.
- Zuckerkindl, F. & Messiner-Klebermass, L. (1931). *Biochem. Z.* **236**, 19.

The Kinetics and Specificities of Deamination of Nitrogenous Compounds by X-radiation

By W. M. DALE, J. V. DAVIES AND C. W. GILBERT
Christie Hospital and Holt Radium Institute, Manchester

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In continuation of previous investigations on the specificity of radiation effects (Dale, 1942, 1947; Allsopp, 1944, 1948) we have found that deamination occurs when aqueous solutions of amino-acids are irradiated by X-rays (Dale & Davies, 1949), a fact which was independently observed by Stein & Weiss (1948). The specificity of this reaction and its peculiar kinetics described in this paper are of consequence with respect to the mode of the indirect action of radiation on solutions and probably for the radiobiology of cells. An attempt will be made to interpret the experimental results in terms of the primary formation of radicals from water by X-rays (Weiss,

1944). It is, however, possible that the current concept of radical formation may need modification in view of certain still unexplained facts, for example, the variation of the protective power of certain substances with their concentration (Dale, Davies & Meredith, 1949).

The approach to the problem of the relation between radiation effect and molecular structure of the solute, presented in this paper, is different from that used previously. Whereas then an overall picture of the radiosensitivity of a substance was indirectly obtained by measuring the competition between two solutes for the available radiation

energy, there is now only one solute present in any one experiment and the formation of ammonia is directly measured in relation to the molecules from which it is derived.

EXPERIMENTAL

Methods. Radiation was obtained either from a 500 kV. Metropolitan-Vickers continuously evacuated X-ray tube (half value layer 5.2 mm. Cu, average $\lambda = 0.056$ Å.) or from a Victor KX 10, 140 kV. tube (half value layer 3 mm. Al, average $\lambda = 0.29$ Å.). The dosage rate at the position of the solution was determined by a 250 r. Victoreen condenser dosimeter which had been calibrated at the National Physical Laboratory. A Hammer dosimeter was used to integrate the total dose given. The general treatment of glassware complied with rules described earlier (Dale, Gray & Meredith, 1949). All solutions were made up by weight with air-saturated water redistilled from alkaline KMnO_4 in an all-glass (Pyrex) still and then exposed to radiation in fused glass ampoules.

Analytical procedure. The NH_3 was distilled in a standard microdiffusion apparatus (Conway, 1947) from a solution half-saturated with respect to K_2CO_3 into standard acid. Usually 1.0 ml. of 0.02N- H_2SO_4 was employed. For the final colorimetric estimation of NH_3 by nesslerization a Spekker photoelectric absorptiometer calibrated for the range of 1–10 $\mu\text{g.}$ of $\text{NH}_3/\text{ml.}$ was used. We are indebted to Adam Hilger and Co. Ltd. for the loan of this instrument. Whenever a greater amount of NH_3 was found in preliminary experiments, the solution under test was immediately diluted with nesslerized water to a final concentration falling within the prescribed range. Dilution was carried out by adding rapidly the required volume of nesslerized water to the distillate contained in a test tube and completing the mixing by pouring the combined fluids back. This procedure helped to avoid interfering turbidity. Only in the few cases in which the solution was strongly alkaline (carboxypeptidase, guanidine carbonate and glyoxaline), was irradiation carried out in the Conway unit itself, the outer ring containing the solution and the central compartment the standard acid. No further alkali was added.

For all experiments blanks were included in order to determine any extraneous traces of NH_3 or any NH_3 which might have been split off from the substances under the conditions of the analysis. Any such blank value was subtracted from the experimental value. Very careful and thorough recrystallization of the amino-acids, etc., is necessary in order to reduce the blanks to negligible values. In a few cases the amounts of amino-acids available for the experiments were not sufficient to permit of a thorough recrystallization and such samples tended to show a high blank.

RESULTS

Variation of ionic yield of glycine with concentration

The X-ray dose for all concentrations was 166,000 r. for aqueous solutions up to near saturation ($2 \times 10^5 \mu\text{g./ml.}$). (The density of this solution is 1.08 and of dry glycine 1.16.) The experimental points are shown in Fig. 1. They fall approximately on a curve given by $I \propto C^n$, where $n = 0.3$, I = ionic yield, defined in this paper as the number of molecules of

ammonia liberated for every 32.5 eV. of X-ray energy adsorbed, and C = concentration of glycine. The curve drawn in the figure is calculated on a theoretical basis (see Discussion and Appendix).

Fig. 1. Ionic and absolute ammonia yield from irradiation of glycine by 166,000 r. Points, experimental; curve, theoretical; arrow, dry glycine.

The arrow in Fig. 1 indicates the yield for dry glycine (2.96). To avoid any loss of ammonia in this case, irradiation took place in a closed system consisting of two glass bulbs fused to a connecting tube which separated the dry glycine from 0.01N- H_2SO_4 . After irradiation the contents were mixed and the ammonia determined.

Table 1. Ammonia yield from amino-acids and other nitrogen-containing compounds in 0.13M-solution after exposure to an X-ray dose of 166,000 r.

Substance	$\mu\text{g. NH}_3/\text{ml.}$
Glycine	8.1
Glycine + 1 equiv. HCl	5.0
Glycine + 18 equiv. NaOH	8.0
Alanine	7.2
β -Alanine	4.3
Histidine	11.8
Histidine monohydrochloride	10.6
Lysine monohydrochloride	3.7
Arginine	7.5
Cystine + 1 equiv. HCl	6.3*
Cystine + 1 equiv. NaOH	19.0*
Methionine	6.6†
Glycine anhydride	1.3
Glyoxaline	3.0
Glycylglycine	12.5
Glycylglycine + HCl	3.8
Leucylglycine	6.6
Diglycylglycine	7.0
Proline	0
Urea	0.2
Guanidine carbonate	0
Thiourea	1.4
[Carboxypeptidase], 2.3%, 6.6×10^{-4} M	9.1*

* Large blank.

† Relatively large blank.

‡ Mol. wt. 32,000 (Putnam & Neurath, 1946); 34,000 (Dale, Gray & Meredith, 1949).

*Deamination of amino-acids and other
nitrogen-containing compounds*

All compounds were irradiated in 0.13M-solutions (equivalent to 10 mg. of glycine/ml.) with an X-ray dose of 166,000 r. The results are summarized in Table 1 and considered in the discussion.

Effect of exclusion of oxygen

Experiments were carried out with oxygen-free solutions of glycine which gave the same yield of ammonia as aerated solutions. In addition, glycine dissolved in solutions of 0.0005–0.002M-hydrogen peroxide gave the same yield of ammonia as glycine dissolved in water. (0.0005M-hydrogen peroxide solution is equivalent to a concentration of peroxide resulting from an X-ray dose of 166,000 r., if it were formed with an ionic yield of unity.)

DISCUSSION

In discussing the results and attempting to fit them into current concepts of the mode of action of ionizing radiations, attention must be paid to the kinetics of the reaction and to the specificity of the radiation effects. The inactivation of enzymes (Dale, Meredith & Tweedie, 1943; Dale, 1943) and viruses (Lea, Smith, Holmes & Markham, 1944) by increasing doses of radiation was shown to follow an exponential course owing to the increasing protection given by the steadily growing fraction of solute, already deactivated, but still capable of reacting with the agent of indirect action formed from water. The radiation effect could only be measured in terms of a change of the initial concentration of the solute, a change which had to become fairly large in order to obtain accurate results. In the present investigation the ammonia split off by radiation is directly measured when only a few parts per thousand of the initial glycine have been changed. One would expect therefore an effect proportional to the dose, and this was verified experimentally.

The ionic yield of the deamination of glycine

The radiation producing deamination of dissolved glycine acts partly directly on the glycine and partly indirectly via the water. The action on dry glycine is direct only and the direct action in solution is proportional to the concentration of the solute. We can apportion the contributions of the direct and indirect action to the ammonia yield in the 18% solution; the direct action will give 3.8 $\mu\text{g./ml.}$ for 166,000 r., being the yield from 1.8 g. of glycine, and the indirect action will therefore contribute 19.9 $\mu\text{g./ml.}$ to give the total ammonia yield of 23.7 $\mu\text{g./ml.}$ In more dilute solutions the proportion due to direct action is even less. These considerations show that the effects are mainly indirect. The experi-

mental results have, therefore, to be discussed in terms of indirect action, the most characteristic feature of which has hitherto been the constancy of the ionic yield when the concentrations of the solute are varied, except at extreme dilutions. The deamination of glycine (Fig. 1) shows no such constancy over the whole range of concentrations from very dilute to nearly saturated solutions, indicating a more complicated mechanism. The usual explanation for the falling ionic yield at extreme dilution is the recombination of the hydroxyl radicals and hydrogen atoms formed from the water. This explanation may hold for deamination, if recombination is still appreciable at the high concentrations, because of a low affinity of the radicals for glycine (Dale, 1942).

The experiments show that the ionic yield rises to a value of 2.9 without any tendency to approach a limit. The ionic yields so far observed for biological substances lie between the limits of 0.1 and 1.0, and thus the deamination of glycine by X-rays seems to be the first example of a non-polymerizing reaction of an organic compound which can greatly exceed unity. (A second example is the liberation of sulphur from thiourea by radiation (Dale & Davies, 1949).)

How then can the high ionic yield both for aqueous solutions and dry glycine be explained? We shall examine two alternative mechanisms to account for it in aqueous solution, one invoking formation of several pairs of radicals per ion pair and the other a chain reaction. It is obvious that the mechanisms to be discussed for the aqueous solutions cannot explain the yield from the dry glycine, and we shall not attempt to specify the way the energy is utilized in this case.

To begin with the non-chain radical mechanism. Weiss (1944) has suggested that the effects of ionizing radiations are due to hydrogen and hydroxyl radicals formed by the splitting of water molecules. It has frequently been assumed that about one effective radical pair is formed for each ion pair (Lea, 1947). On the basis of this assumption an ionic yield exceeding unity could not primarily occur, since one radical by its very nature cannot react with more than one molecule of glycine. However, radicals may be formed by excitation or direct dissociation as well as by neutralization of charged ions (Dainton, 1948; Miller, 1948). Miller suggests that, in the case of his inorganic solute, a total of about three pairs of radicals are formed per ion pair (or, more exactly, per 32.5 eV. of energy absorbed from the radiation). This is energetically possible, since it requires 5 eV. to form a pair of radicals and from the 32.5 eV. absorbed only about 12 eV. is needed to ionize the water molecules. If both radicals are effective (Dale, 1947, p. 49) in reacting with the solute either directly or indirectly by a mechanism such as suggested by Miller (1948), then an ionic yield as high as 6 could be obtained without any form of chain reaction.

The other alternative is a chain reaction. Dainton (1948) has recently shown that hydroxyl and hydrogen radicals catalyse the polymerization of vinyl cyanide and 2-cyanoprop-1-ene. In this reaction the radical is initiating a chain reaction which can continue without further participation of a radical of the same type. For the deamination of glycine, however, it is unlikely that after a hydroxyl radical has collided with a glycine molecule an ammonia formation could progress on its own without further radicals taking part. A mechanism on the following lines might be suggested:

The re-formed hydroxyl radical can then collide with another glycine molecule and so carry on the chain.

For the deamination of amino-acids by ultra-violet light (Weizmann, Bergmann & Hirschberg, 1936) an ionic mechanism was proposed on account of a maximum quantum efficiency for the isoelectric state of the amino-acid examined. This mechanism does not seem to apply to the deamination of amino-acids by X-rays since the hydrochlorides of amino-acids yield less ammonia than the amino-acids themselves (Table 1).

The variation of ionic yield with concentration of solute

The schemes suggested above give a broad picture of how ionic yields exceeding unity may occur. They do not yet account for the particular form of the variation of yield with concentration which can be explained by considering the kinetics of the reaction and the spatial distribution of the radicals. The energy of the X-radiation is transferred to electrons (Compton recoil or photoelectrons), and the radicals are formed initially along their tracks, either from ions or from water molecules directly excited or dissociated by the electron. The initial density of the radicals is high, and therefore only at high concentrations of solute will their recombination be negligible. The rate of loss of radicals can be represented by the equation

$$-\frac{dn}{dt} = -D \cdot \nabla^2 n + \alpha n^2 + \beta Cn,$$

where n is the concentration of radicals, C the concentration of the solute, D the diffusion constant of the radicals, α the velocity constant of the recombination of the radicals, β the velocity constant for the interaction of the radicals and solute leading to the removal of the radical and t the time.

At the dose rates used the highly localized formation of radicals along the electron paths confines the entire reaction to their immediate neighbourhood, so that in the equation above we need only consider

one electron track, and therefore the reaction is independent of dose rate. The initial concentration distribution of radicals has a definite value n_0 , and their subsequent fate is described by the solution of the equation. On the basis of this equation a curve covering both mechanisms has been calculated (see Appendix), the numerical values of the parameters having been chosen to give the best fit to the experimental points (Fig. 1). It is worth noting that the choice of constants is not critical and in particular that no reliable estimate can be made, in the case of the non-chain reaction, of the number of effective radicals per ion pair and in the other case of the chain length.

The peculiarity of the deamination reaction by X-rays is brought out on comparing this reaction with the inactivation of enzymes and viruses (Dale, 1947; Lea, 1946), where the recombination of radicals becomes negligible at much lower concentrations, resulting in a constant ionic yield over an appreciable range of concentrations. The deamination, in fact, never attains constancy of its ionic yield, and it is uncertain whether such a state would be reached if higher concentrations were physically possible. This uncertainty makes a rational interpretation more difficult and one can only tentatively interpret the different behaviour of the various substances being deaminated in terms of their respective affinity to radicals (considerably lower than for enzymes). This difficulty becomes even greater in the liberation of sulphur from thiourea (Dale & Davies, 1949) which follows a similar course, although protection experiments suggest a high affinity (Dale, 1947).

In the present state of knowledge one is justified in attempting an interpretation in terms of radicals and their recombination, but, as pointed out in the introduction, present views of the mechanism discussed in this paper may have to be modified in the future.

The specificity of the deamination of amino-acids and other nitrogen-containing compounds

As is shown in Table 1 the α -amino-acids—glycine, alanine and arginine—are deaminated to nearly the same extent, whereas histidine exceeds the average value. This larger value for histidine may either be due to a contribution from the glyoxaline part of the molecule, since glyoxaline itself yields a certain amount of ammonia on irradiation, or to a weakening of the strength of the C-N bond in the α -amino group through the vicinity of the glyoxaline or to both these causes. An amino group in the β -position (β -alanine) appears to be more resistant to deamination. In the case of cystine and methionine, deamination takes place, but no further conclusions are drawn since the blank values were too high. When an equivalent amount of alkali is added to

glycine deamination remains at the normal level; however, a most striking effect on deamination is observed when an equivalent amount of acid is added to the solution of amino-acids and dipeptides. The ammonia yield then falls by about one third. Apparently the vicinity of the undissociated carboxyl group, which in the dissociated state shows a marked resistance to radiation (Dale, 1947), affects the strength of the C-N bond. The effect is masked in the case of histidine where the hydrochloric acid combines mainly with the basic group of the glyoxaline. The combination of two glycine molecules in ring form (glycine anhydride) considerably reduces the ammonia value, and similarly the peptide linkage in leucylglycine and diglycylglycine does not contribute to deamination, but glycylglycine appears to be an exception yielding more ammonia than glycine. The imino group in proline and the amino groups, combined with carbon dioxide in guanidine carbonate or free in urea and thiourea, are either completely inert or very nearly so.

In the experiments with crystalline carboxypeptidase, representing a highly purified protein, large blanks were obtained and the value of 9.1 μg . of NH_3 /ml. given in Table 1 is therefore not very reliable.

Biological significance

From the biological point of view the high ionic yield of deamination may be of importance. The deamination of amino-acids and proteins in vital regions is likely to have a toxic effect on cell functions and their co-ordination. It has been shown (Auerbach & Robson, 1947; Lobashov, 1937) that ammonia vapour has a slight, but definite, mutational effect in *Drosophila*. The permeation of ammonia from outside to vital centres can only be incompletely achieved and the concentrations used experimentally are limited by toxic effects on the whole organism. Radiation, however, will generate ammonia in these centres themselves and thereby produce a higher concentration than can possibly be achieved by diffusion from outside.

APPENDIX*

Calculation of ionic yields

In calculating the ionic yield of the deamination process we need only take account of radicals formed along a single electron track. We consider one type of radical only and suppose that they diffuse

* Note added in proof. Dr C. B. Allsopp recently sent us a manuscript by the late Dr Lea and himself, drafted in 1944, arriving at identical conclusions. The paper was not published because of insufficient experimental data at that time, but formed the basis of Fig. 4, p. 58, in Lea's book (Lea, 1946). We thank Dr Allsopp for encouraging us to publish our appendix in its present form.

away from their positions of formation until they are removed from the solution by either recombination with another radical or by interaction with the solute. We suppose that the radicals are initially distributed in a column along the electron tracks in a manner similar to the charged ions so that the initial density of radicals at distance r from the axis of the column is given by the following equation (Jaffé, 1913; Kara-Michailova & Lea, 1940; Lea, 1947):

$$n = \frac{N_0}{\pi b^2} e^{-r^2/b^2},$$

where N_0 is the total number of radicals per unit length of track, and b a constant giving a measure of the initial radius of the distribution.

The rate of loss of concentration of radicals is given by

$$-\frac{dn}{dt} = -D \cdot \nabla^2 n + \alpha n^2 + \beta Cn,$$

where C , D , α and β have the meaning as defined

above; $\beta = \frac{Zp}{M}$, where Z is the collision frequency

between a radical and a solute molecule, p is the probability that a reaction occurs at any collision between radical and solute, and M is the average chain length, so that there is a chance of $1/M$ that the radical is lost per encounter; for a non-chain reaction $M=1$. If diffusion only takes place, the distribution of radicals remains gaussian, and we make the assumption, following Jaffé (1913), that the distribution also remains gaussian when loss of radicals occurs due to recombination and capture, though this distribution is not an accurate solution of the differential equation. Writing, therefore,

$$n = \frac{N}{\pi (4Dt + b^2)} e^{-r^2/(4Dt + b^2)},$$

where N is the total number of radicals per unit length of track remaining at time t , we can substitute this value and integrate over the column to get

$$-\frac{dN}{dt} = \int_0^\infty (\alpha n^2 + \beta Cn) 2\pi r dr = \frac{\alpha N^2}{2\pi (4Dt + b^2)} + \beta CN.$$

The total number of molecules of ammonia formed per unit length of track will be

$$p_1 \int_0^\infty ZpCN dt,$$

where p_1 is the probability of liberation of ammonia following reaction with a radical.

If we take the initial number of ion pairs per unit length of track to be N_0/R (i.e. R radicals per ion pair) then the ionic yield I is given by

$$I = p_1 R \int_0^\infty ZpC \frac{N}{N_0} dt.$$

We now have to solve N as a function of time.

We have so far considered one type of radical only, but if two radicals (H and OH) are present, both of which react with the solute, the same equations are obtained, if we assume that the total numbers of both types decrease at the same rate, though they may have different spatial distributions. For the constants D and b^2 we now use the mean values of those for each radical and for α the total velocity constant for recombination by all processes.

To solve the equations we make the following changes of variables:

$$y = N/N_0, \quad x = \frac{4D}{b^2} t + 1,$$

and write
$$P = \frac{\alpha N_0}{8\pi D},$$

and
$$Q = \frac{\beta b^2}{4D} C = \frac{1}{M} \frac{Zpb^2}{4D} C.$$

The differential equation then becomes

$$-\frac{dy}{dx} = P \frac{y^2}{x} + Qy,$$

with the initial condition $y = 1$ when $x = 1$, and

$$I = p_1 RMQ \int_1^\infty y dx.$$

The differential equation is a Riccati equation (see Piaggio, 1933) and can be solved by making the substitution

$$y = \frac{x}{Pu} \frac{du}{dx},$$

giving
$$\frac{d^2u}{dx^2} + \left(\frac{1}{x} + Q\right) \frac{du}{dx} = 0.$$

This gives
$$\frac{du}{dx} = \frac{A}{x} e^{-Qx},$$

where A is a constant, and therefore

$$u = A \int_1^x e^{-Qx} \frac{dx}{x} + B,$$

where B is another constant. We therefore get

$$\frac{1}{y} = \frac{P}{x} \frac{A \int_1^x e^{-Qx} \frac{dx}{x} + B}{\frac{A}{x} e^{-Qx}} = P e^{Qx} \int_1^x e^{-Qx} \frac{dx}{x} + e^{Q(x-1)},$$

since $y = 1$ when $x = 1$.

Write

$$-\text{Ei}(-x) = \int_x^\infty e^{-x} \frac{dx}{x} \quad 0 < x \quad (\text{exponential integral}).$$

Then
$$\frac{1}{y} = P e^{Qx} [\text{Ei}(-Qx) - \text{Ei}(-Q)] + e^{Q(x-1)}.$$

A further integration gives the ionic yield

$$I = p_1 RMQ \int_1^\infty \frac{e^{-Qx} dx}{P [\text{Ei}(-Qx) - \text{Ei}(-Q)] + e^{-Q}}.$$

Make a change of variable

$$z = e^{-Q(x-1)},$$

write

$$s = e^{-Q},$$

and

$$\text{Li}(x) = \int_0^x \frac{dx}{\log x} \quad 0 \leq x < 1 \quad (\text{logarithmic integral}),$$

so that

$$\text{Ei}(-x) = \text{Li}(e^{-x}),$$

and

$$\text{Li}(x) = \text{Ei}(-\log 1/x),$$

then
$$I = p_1 RM \int_0^1 \frac{dz}{1 + \frac{P}{s} [\text{Li}(sz) - \text{Li}(s)]}.$$

The exponential integral is a tabulated function and the last substitutions were designed to get the ionic yield in a form so that the final integration can be carried out most conveniently. The integration is taken between definite limits 0 and 1, and the integrand is a monotonic function increasing to 1 at $z = 1$.

The relation between the ionic yield and the concentration of solute is seen to contain three parameters, P and constants multiplying I and C respectively, and their values must either be known or chosen to give the best fit with the experimental results. In the absence of any reliable data for the constants which are combined to form the parameters, we have to adopt the latter alternative.

The full line in Fig. 1 has been calculated using the following values for the parameters:

$$P = 2.0, \quad p_1 RM = 6.0, \quad \frac{1}{M} \frac{Zpb^2}{4D} = 2.24 \text{ ml./g.}$$

The choice of P is fairly critical, values of 1 or 5 giving definitely worse results. It is of interest to compare this value with that calculated from

$$P = \frac{\alpha N_0}{8\pi D},$$

taking $\alpha = 4 \times 10^{-10} \text{ cm}^3 \text{ sec}^{-1}$

and $D = 4.5 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1},$

the mean of the H and OH diffusion constants (see Lea, 1947). We can assume 10^6 ion pairs per cm. of electron track and therefore the number of radical pairs per cm. N_0 , is $R \times 10^6$. The value of P is then 2.1 if we take six effective radicals per ion pair, or 0.35 if we take one. Since the constants can only be estimated approximately the agreement is satisfactory, and the shape of the experimental curve is therefore consistent with the recombination hypothesis.

The high ionic yield can be accounted for equally well by assuming either about six effective radicals (three pairs) per ion pair, or a chain reaction with an effective chain length of six, or by a combination of the two. This is on the assumption that p_1 is 1,

smaller values of p_1 would require larger value of R or M . One can only try to estimate the value of b , the initial radius of the column of radicals, by analogy with the results of ion recombination in gases. From these we would infer that we should use a value of about 2×10^{-7} cm., the value is low because we are using a columnar theory, whereas the ions (and probably also the radicals) are formed in clusters along an electron track (see Kara-Michailova & Lea, 1940). Using this value for b and $Z = 4 \times 10^{-10}$ cm.³sec.⁻¹, we have to assume $p = 0.003$ for the non-chain reaction, or $p = 0.019$ for the chain reaction, both cases therefore giving a low affinity of the radicals for the solute.

SUMMARY

1. Experiments are described showing the deamination by X-rays of some amino-acids and their derivatives, polypeptides and related nitrogenous

compounds. The relationship between the ionic yield and radiation dose has been studied in detail for glycine. The ionic yield rises from 0.3 to 2.9 for very dilute to nearly saturated solutions. The ionic yield for dry glycine was 2.96.

2. An explanation of the high ionic yield with glycine is attempted in terms of the radical hypothesis assuming either a chain reaction or the formation of radicals by excitation in addition to charge neutralization. The continuous rise of the yield is interpreted as being caused by recombination of radicals competing with deamination even at the highest concentrations of glycine, due to a low affinity of glycine to radicals. The explanation offered, although consistent with the experiments described, is not wholly satisfactory in view of the radiation effects on thiourea.

3. The biological significance of the results is discussed.

REFERENCES

- Allsopp, C. B. (1944). *Trans. Faraday Soc.* **40**, 79.
 Allsopp, C. B. (1948). *Brit. J. Radiol.* **21**, 242.
 Auerbach, C. & Robson, J. M. (1947). *Proc. Roy. Soc. Edinb. B*, **62**, 284.
 Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, 2nd ed. London: Crosby, Lockwood and Son, Ltd.
 Dainton, F. S. (1948). *J. phys. colloid. Chem.* **52**, 490.
 Dale, W. M. (1942). *Biochem. J.* **36**, 80.
 Dale, W. M. (1943). *Brit. J. Radiol.* **16**, 171.
 Dale, W. M. (1947). *Brit. J. Radiol. Suppl.* No. 1, 46.
 Dale, W. M. & Davies, J. V. (1949). *Nature, Lond.*, **163**, 64.
 Dale, W. M., Davies, J. V. & Meredith, W. J. (1949). *Brit. J. Cancer*, **3**, 31.
 Dale, W. M., Gray, L. H. & Meredith, W. J. (1949). *Philos. Trans. A*, **242**, 33.
 Dale, W. M., Meredith, W. J. & Tweedie, M. C. K. (1943). *Nature, Lond.*, **151**, 280.
 Jaffé, G. (1913). *Ann. Phys., Lpz.*, **42**, 303.
 Kara-Michailova, E. & Lea, D. E. (1940). *Proc. Camb. phil. Soc.* **36**, 101.
 Lea, D. E. (1946). *Action of Radiations on Living Cells*. Cambridge: University Press.
 Lea, D. E. (1947). *Brit. J. Radiol. Suppl.* No. 1, p. 59.
 Lea, D. E., Smith, K. M., Holmes, B. & Markham, R. (1944). *Parasitology*, **36**, 110.
 Lobashov, M. E. (1937). *Genetics*, **19**, 200.
 Miller, N. (1948). *Nature, Lond.*, **162**, 448.
 Piaggio, H. T. H. (1933). *Differential Equations*. London: Bell.
 Putnam, F. W. & Neurath, H. (1946). *J. biol. Chem.* **166**, 603.
 Stein, G. & Weiss, J. (1948). *Nature, Lond.*, **162**, 184.
 Weiss, J. (1944). *Nature, Lond.*, **153**, 748.
 Weizmann, C. H., Bergmann, E. & Hirschberg, Y. (1936). *J. Amer. chem. Soc.* **58**, 1675.

An Investigation of the Intracellular Fluid of Calf Embryo Muscle

By A. H. GORDON, *Biological Institute of the Carlsberg Foundation, Copenhagen*

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Van Slyke (1913) estimated the amount of amino nitrogen extractable with boiling water from the skeletal muscle of the dog. Very few attempts have, however, been made to estimate or even to identify the particular amino-acids present in the intracellular fluid of any tissue. The work on muscle has been summarized by Ussing (1943), who mentions estimations of arginine, histidine, cystine, glutamic acid, tyrosine and leucine plus valine. Since then Christensen, Streicher & Elbinger (1948) have

estimated glycine and glutamine in guinea pig muscle.

The present investigation concerns the substances in calf embryo muscle with molecules small enough to pass through a dialysis membrane; these mixtures are also compared with similar ones obtained from adult ox muscle and serum. Embryo muscle was used because extracts of this material are employed to maintain the growth of tissue cultures; although the stimulating effect of embryo extracts may be due