PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

The Inactivation of an Enzyme (Carboxypeptidase) by X- and $ \alpha $ -Radiation

W. M. Dale, L. H. Gray and W. J. Meredith

Phil. Trans. R. Soc. Lond. A 1949 **242**, 33-62
doi: 10.1098/rsta.1949.0004

THE INACTIVATION OF AN ENZYME (CARBOXYPEPTIDASE) BY X- AND α-RADIATION

By W. M. DALE,* L. H. GRAY† and W. J. MEREDITH*

(*Communicated by E. K. Rideal, F.R.S.—Received* 17 *May* 1947—*Revised* 21 *August* 1948)

The inactivation of aqueous solutions of pure crystalline carboxypeptidase by X-radiation and α-radiation has been studied. It is concluded that in each case the enzyme molecules are inactivated by collision with a labile product resulting from the ionization of the water. The X-ray ionic yield is approximately constant and equal to about 0·18 enzyme molecules inactivated per ion pair of water at all enzyme concentrations between about 2×10^{-4} and 2×10^{-1} g. enzyme/ml. At lower concentrations a smaller X-ray ionic yield was observed. At an enzyme concentration of 3×10^{-5} g./ml. the results are not affected by the absence of dissolved oxygen. The α-ray ionic yield is only about one-twentieth of the X-ray ionic yield, that is, ~0·01 molecules of enzyme inactivated per ion pair of water and appears to increase about twofold over the concentration range 5×10^{-6} to 5×10^{-3} g. enzyme/ml. An attempt is made to correlate the X-ray and α-ray ionic yields with what is known concerning the spatial distribution of the ions. It is concluded that the extremely high concentration of positive ions which forms the core of the α-ray track probably plays a very significant role in the general radiochemistry of α-radiation. The small inactivation of carboxypeptidase brought about by exposure to α-rays could be ascribed to the secondary electrons (δ-rays) which travel clear of the α-ray track and it is not certain that any inactivation of enzyme is brought about by the primary α-ray ionization.

Introduction

When carboxypeptidase in dilute aqueous solution is exposed to X-radiation, the specific hydrolyzing power of the enzyme towards substrates such as a peptic digest of edestin or chloracetyltyrosine is reduced. At a given enzyme concentration the residual activity is an exponential function of the dose and the fact that over a wide range of enzyme concentrations the exponent is inversely proportional to concentration has been interpreted as indicating that the enzyme molecules are not directly affected by the ionizing radiation but indirectly through collision with a labile product, resulting from the ionization of the water. This interpretation receives support from various other circumstances of the inactivation (Dale 1940, 1942, 1943; Dale, Meredith & Tweedie 1943). The precise nature of the change in the enzyme molecule responsible for the loss of activity is unknown, and, although it has been plausibly suggested (Weiss 1944) that the ionization of water leads directly to the production of atomic hydrogen and hydroxyl radicals, it has not so far been demonstrated experimentally that these radicals play a part in this particular reaction. It was thought to be of interest, as possibly throwing some light on these questions, to compare the effects of X-radiation with those of another ionizing radiation which gives rise to a very different spatial distribution of ions, and for this purpose α-radiation is convenient.

* Christie Hospital and Holt Radium Institute, Manchester.

† M.R.C. Radiotherapeutic Research Unit, Hammersmith Hospital, London. The experimental work was carried out while Dr Gray was at the Mount Vernon Hospital, Northwood, Middlesex, and working under a grant from the British Empire Cancer Campaign.

Cameron & Ramsay (1907*a*, *b*, 1908) demonstrated the decomposition of water by α-radiation. The very careful experiments of Duane & Scheuer (1913) established that pure air-free water is decomposed by α-radiation with approximately unit ionic efficiency—that is, approximately one molecule of water is decomposed for each molecule ionized. A small amount of hydrogen peroxide was also produced. Nurnberger (1936) reported the decomposition of air-free water with accompanying production of hydrogen peroxide and estimated his ionic yield to be approximately unity, though his published data appear to indicate a considerably smaller yield.* Lanning & Lind (1938) also found that α-rays decompose water with approximately unit ionic efficiency and, from the circumstances of their experiments, it would appear that the water was initially air-free. There seems, therefore, no doubt that pure initially gas-free water is decomposed by α-radiation with approximately unit ionic efficiency and that, with the doses employed, some hydrogen peroxide is produced.† There is, on the other hand, some doubt regarding the extent to which pure gas-free water is decomposed by X-radiation. The evidence has been reviewed by Allsopp (1944), Lea (1946), and more recently by Dainton (1948).

It seems well established (Frilley 1947) that when pure water, saturated with air at atmospheric pressure, is exposed to small doses of X-radiation, the dissolved oxygen is reduced to hydrogen peroxide with ionic efficiency approaching unity. Bonet-Maury & Lefort (1948) find that the ionic yield of hydrogen peroxide is the same when oxygenated water is irradiated by α-radiation as when irradiated by X-radiation. Lanning & Lind (1938) found that fairly strong solutions (0·1 to 1 N) of hydrogen bromide, hydrogen iodide and potassium permanganate were decomposed by α-radiation with ionic efficiency of the order of unity.

With the possible exception of the decomposition of water, therefore, these results exhibit no striking difference between the effects of X-radiation and α-radiation on water or aqueous solutions. One such difference appears in the literature, namely the effect on tyrosine. Stenstrom & Lohmann (1928) found that tyrosine was decomposed by X-radiation with ionic efficiencies $M/N = 0{\cdot}166$ and $0{\cdot}08$ at concentrations of 10^{-3} g./ml. and 2×10^{-5} g./ml. respectively, whereas Nurnberger (1937) found that it was decomposed very slightly, if at all, by α-radiation ($M/N < 0{\cdot}003$).‡ In the present experiments the inactivation of carboxypeptidase by α-radiation has been found to be 20 times smaller than that produced by X-radiation. Svedberg & Brohult (1939) studied the effect of α-radiation on solutions of *Helix* haemocyanin, haemoglobin and serum albumin at room temperature, and also frozen at the temperature of liquid air. The results exactly paralleled the effect of exposing these proteins to ultra-violet light. No experiments with X-rays were reported. The haemocyanin was split into half-molecules, regardless of temperature, while haemoglobin and serum albumin were made inhomogeneous by the formation of a continuous series of molecules of both lower and higher molecular weight when irradiated at room

* D. E. Lea (1946, p. 41). We concur with this view.

† Very recently Bonet-Maury & Lefort have observed that hydrogen peroxide is formed both in gas-free and in oxygenated water by α-radiation with an ionic efficiency of about one-sixth. Other experiments by these authors throw light on the decomposition of water by X-radiation and α-radiation referred to in the following paragraph (see Discussion, p. 51).

‡ It would seem that the whole effect observed by Nurnberger could be ascribed to β-rays accompanying the α-rays.

temperature, but not when irradiated at liquid-air temperature. Quantitative data were only given for the splitting of haemocyanin. The number of molecules remaining unsplit was an exponential function of the dose, but as data are only given for one concentration ($\sim 10^{14}$ molecules/ml. or $\sim 1{\cdot}1 \times 10^{-3}$ g. protein/ml.) it is not possible to apply the usual criteria for discrimination between direct and indirect action. Since the number of molecules split was approximately equal to the number actually traversed by an α-particle it is likely that the direct mechanism was operative under the condition of the experiments. The results are therefore not strictly comparable with those to be reported concerning the inactivation of carboxypeptidase.

The experiments with carboxypeptidase are of biological as well as chemical interest, since there could be little doubt that some of the biological effects of ionizing radiations derive from chemical changes produced in the aqueous phase in the cells. Comparison between the influence of ion density in chemical and biological effects should therefore help to reveal correlation between the two. The influence of linear ion density on virus inactivation, gene mutation, disinfection of bacteria, the inhibition of mitosis, the production of chromosome structural changes, the retardation of growth and the lethal effects of radiation on a variety of normal and malignant tissues has been studied already. The subject has recently been reviewed by Gray (1946).

An analysis of the effects of various radiations should start from the simplest systems possible. A homogeneous solution of some substance which allows a ready and reliable response to irradiation to be measured quantitatively constitutes such a simple system, and is in marked contrast to inhomogeneous biological systems. The advantages of using solutions of crystalline enzymes as sensitive indicators of radiation effects have already been shown (Dale 1940, 1942).

This paper reports an attempt to compare the effects of X- and α-radiations on solutions of the crystalline enzyme carboxypeptidase, which has the necessary qualities of stability, high activity and suitable reaction kinetics.

Biochemical technique

General remarks

The determination of enzymatic activity was carried out with chloracetyltyrosine as substrate, according to Anson (1937*b*), and the volume of the enzyme solution and the length of the digestion time were adjusted in accordance with the principles of a previous investigation (Anson 1937*b*; Dale 1940). The definition of enzyme-protein concentration was derived from chemical analysis (nitrogen content $\times$ 6·94 = protein content) (Anson 1937*a*) of a standard suspension of crystals and all dilutions were based on this value. A definition of the concentration of enzyme protein by measuring enzyme units would lead to deviation from the actual protein concentration, since dilution of the enzyme solution by volume and dilution by units are not strictly proportional (Dale 1940).

The disproportionality only shows in the range of extreme dilutions where this enzyme, in common with some others, is less stable and some fluctuation of its activity occurs. The activity of carboxypeptidase decreases more rapidly than its concentration at the lowest

concentrations (Dale 1940). Further experiments* have shown that this is also true when the substrate is chloracetyltyrosine instead of a peptic digest of edestin.† Since in our experiments the activity of an irradiated solution was always compared with that of a sample of the same enzyme solution which had not been irradiated, the lack of proportionality between enzyme activity and enzyme concentration could at most have only slightly decreased the estimated inactivation dose. The result of experiments in which a given enzyme solution was exposed to a range of doses indicates, however, that this was not the case under the conditions of our experiments. When the inactivation doses of enzyme solutions of different concentration are compared, the greater instability at very great dilution leads to a scatter of the observations about a smooth curve which is larger than occurs for more concentrated solutions, as shown later in the statistical section.

In view of the special experimental requirements obtaining for radio-chemical problems, it seems important to stress certain experimental details which have to be observed in order to achieve the highest degree of accuracy.

(1) *Titration.* A daylight mercury discharge tube was used as light source instead of natural daylight to ensure constancy of colour matching.

(2) *Dissolving the enzyme.* The suspension of crystals was thoroughly shaken in order to distribute the fine crystals evenly. The required amount was taken out immediately, before the crystals had time to settle, diluted with 'alkaline water' (5 ml. of 0·02 N-NaOH in 500 ml. of glass-redistilled water) to the desired strength and 0·1 N-NaOH added drop by drop, pausing for about 3 min. after every addition and constantly swirling the flask until all crystals were dissolved and the reaction was pink to phenolphthalein. Solution was complete in about 15 min.

(3) Enzyme and substrate solutions were dissolved as late as possible to avoid unnecessary standing, and all titrations were performed as closely together as possible. The substrate solution was filtered before use.

(4) *Treatment of suspension of crystals.* No trace of preservative (e.g. toluene) should be present in the enzyme solution. Stock suspensions of crystals can be kept in a refrigerator at about +4° C with a drop of toluene for a very long time (years), but the suspension used in a series of experiments should be kept in the refrigerator without a preservative. If contamination has occurred or if it is necessary to remove toluene, the suspension of crystals has to be centrifuged in sterile tubes and washed by centrifugation several times with redistilled sterile water. It is advisable to recrystallize the crystals as a routine once in 12 months.

(5) *Treatment of glassware.* Strict avoidance of any contamination with organic matter is necessary because impurities may 'protect' the enzyme from the effect of radiation (Dale 1942). All glassware to be used in the experiment has to be cleansed in a warm concentration of sulphuric acid-nitrate mixture, and after rinsing with tap water, distilled water, and finally glass-redistilled water, is put into a drying oven. Pipettes are slipped into similarly cleaned test-tubes and dried in this position. They are only removed from

* Dale, unpublished.

† This result does not emerge from Anson's work, since, in Anson's curve for a 10 min. digestion time, the range of high dilutions is on too small a scale. Anson's experiments show a deviation from proportionality in the opposite sense, i.e. the activity increases less rapidly than the concentration.

the protecting tubes directly before use and not laid down on the bench without their glass-cover.

(6) All solutions are made up with glass-redistilled water. From the method of preparation, it is evident that the solutions used for irradiation were initially air-saturated. Comparison experiments with an air-free solution of concentration 3×10^{-5} g. enzyme/ml. showed no significant influence of dissolved oxygen on inactivation dose (cf. p. 40).

The source of α-radiation

In all the experiments performed, the irradiation technique was essentially the same; the source of α-particles was dissolved in the enzyme solution and then, after a given exposure, either the source was removed or the effect of the radiation stopped by the addition of substrate; Dale (1940) has shown that the effect of X-radiation on a carboxypeptidase solution is very small indeed in the presence of its substrate (edestin or chloracetyltyrosine). Special experiments were performed which showed that the effect of α-radiation was also eliminated by the presence of chloracetyltyrosine. Initially radon was used as the source of α-particles, then thorium X was tried, and finally a return made to radon.

If a bulb containing radon is broken in a vessel filled with enzyme solution a dose of radiation will be delivered to the solution from the radon and its decay products in the following series:

$$\mathrm{Rn} \xrightarrow{\alpha} \mathrm{Ra\,A} \xrightarrow{\alpha} \mathrm{Ra\,B} \xrightarrow{\beta\gamma} \mathrm{Ra\,C} \xrightarrow{\beta\gamma} \mathrm{Ra\,C'} \xrightarrow{\alpha} \text{etc.}$$

active deposit of short life

From this, it will be seen that the active deposit contributes β- and γ-rays as well as α-particles. The effect of these rays must either be eliminated or reduced to a minimum and allowance made for their effect. Only the β-rays need be considered, as the size of the vessels used was such that the effect of the γ-rays could be neglected.

The total dose received by the solution may be considered as being made up of three parts:

(1) Radiation from the radon released into the solution and from its decay products laid down there.

(2) Radiation from the active deposit on the splinters of the broken bulb.

(3) Radiation from the active deposit remaining in the solution after the removal of the radon.

(2) and (3) are considered as operative up to the moment of adding substrate to the enzyme solution.

In the earliest experiments, a glass bulb containing radon in equilibrium with its 'short-life' decay products was broken in the enzyme solution and exposures of the order of 30 min. used. These experiments showed that the ionic efficiency of α-radiation was very low; indeed, in some cases the whole effect observed could be ascribed to the β-rays alone. Therefore, a reduction in the dose contributed by the β-particles was essential and it was decided to use thorium X instead of radon, since thorium X can be obtained in solution practically free from its decay products if a somewhat lengthy series of chemical manipulations are undertaken.

Unfortunately, however, the thorium X preparation affected the enzyme chemically as well as through its radiations and the method was abandoned after a few experiments. These, however, were of value as showing that inactivation of enzyme was, in fact, brought about by α-radiation, though with low ionic efficiency. The experiments are described in the appendix.

Reverting to radon as the parent α-ray source, an attempt was made to remove the glass splinters (the active deposit on which contributes a considerable portion of the β-ray dose) from the main solution by using a bottle with a long 'tail' (figure 1*a*) into which the

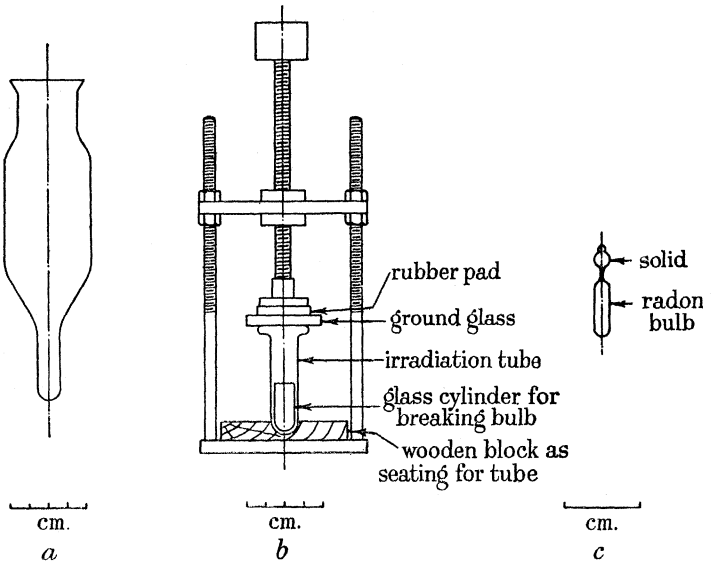


FIGURE 1. Apparatus used for the irradiation of enzyme by α-particles. (*a*) Glass bottle with tail. (*b*) Glass bottle without tail in position for irradiation. (*c*) Radon bulb.

majority of the splinters were shaken, leaving the main solution initially comparatively free from active deposit. For the chemical determinations only solution from the main vessel (and therefore out of the range of the β-particles in the 'tail') was taken. γ-ray measurements made with the 'tail' surrounded by 3 in. of lead indicated that about three-quarters of the splinters had been shaken down. This experiment indicated that the percentage efficiency of α relative to X-rays (η_x^α) was about 5%. It was then decided to extend the measurements to a range of enzyme concentrations and to attempt a further reduction of the β-ray contribution by the use of shorter exposures with larger concentrations of freshly prepared radon. With the sources available, ~100 millicurie (mC), the required concentration could only be achieved by a reduction in the volume of solution irradiated, and this necessitated abandoning the 'tail' type of bottle. In some cases the radon was dissolved in the enzyme solution within 3 min. of being pumped from the stock radium, and the experiment was complete in 23 min. By these means the β-ray inactivation was reduced to about 15% of the α-ray inactivation.

IRRADIATION TECHNIQUES AND MEASUREMENTS

The irradiation vessel is shown in figure 1*b*. It contains a glass cylinder of diameter 4 mm. less than the internal diameter of the irradiation tube, rounded at one end to fit the end of the vessel and flat at the end nearest the top plate, which was used to break the radon

bulb. The tube is made of fairly thick glass, and the top flange and covering glass plate were carefully ground flat with finest carborundum powder. The tube was filled with enzyme solution, the freshly prepared radon bulb dropped in and the ground plate slipped on to the top. Great care was taken to see that no air was trapped in the tube, since the amount of radon per unit volume of air is much greater than that per unit volume of solution with which it is in equilibrium. The whole unit was then carefully clamped with the type of clamp shown in figure 1*b*. One or two shakes were sufficient to smash the bulb and release the radon into the solution. All radon bulbs were of pyrex, since it was found that pyrex glass breaks more readily than soda glass. The shape of the bulb is shown in figure 1*c*—the solid glass end counteracted a tendency to float and made breaking easier.

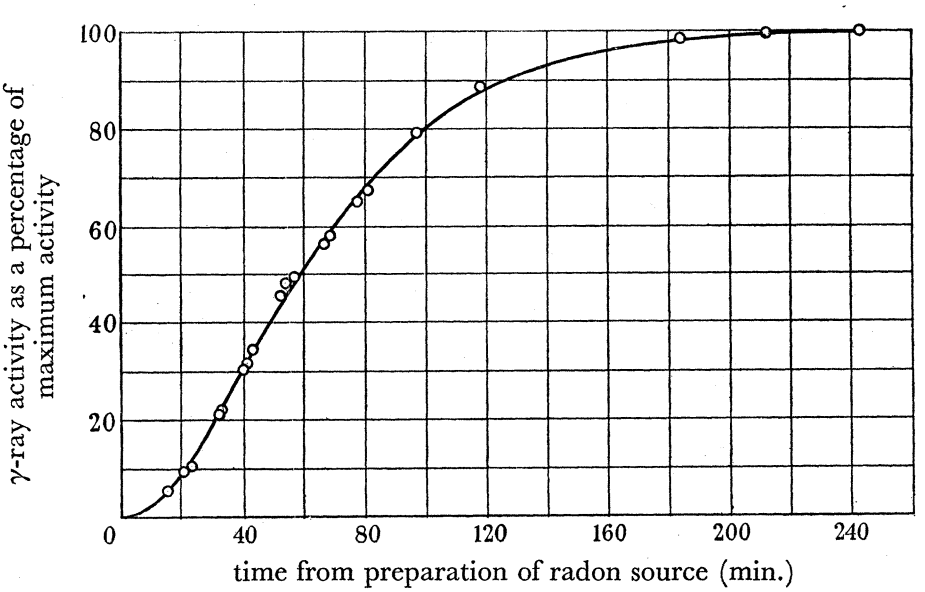


FIGURE 2. The growth of activity of radon preparations.

A similar technique was also employed in the earlier radon experiments, except that glass marbles were used in the larger vessels instead of a cylinder. At an early stage it was shown experimentally that no radon leaked out when the flange and plate were carefully ground, and the vessel and plate carefully clamped together. Thus grease was unnecessary and the dangers of contaminating the enzyme and of radon absorption by the grease were eliminated. The amount of radon used was estimated from the γ-ray activity of the solution, measured on several occasions during the experiment at known times after the preparation of the bulb,* that is, when the activity was still growing. The calibration curve was determined in a separate experiment and checked well with that computed from the radioactive constants, as shown in figure 2. In most enzyme exposures the bulb was broken within 4 min. of its preparation, and the exposure was of the order of 20 min. Separate tests were made to ascertain with what accuracy the equilibrium γ-ray activity could be inferred from measurements made within the first 20 min. after the preparation of the source (table 1). The results show that the standard error in the estimation of radon concentration by this procedure is $\sim\pm 4\,\%$.

* This was taken to be 30 sec. before the bulb was sealed off.

TABLE 1

time after preparation of tube (min.)	estimated value (mC)	full activity value (mC)	percentage error
20	46·7	45·9	+1·8
40	38·4	39·2	−2·1
15	20·6	20·0	+3·0
8	50·0	46·5	+7·0
30	30·6	31·3	−2·3

At the end of the exposure time the top plate was removed in the open air and the fluid poured into a Petri dish to form a layer about 2 mm. deep, over which flowed for 5 min. a rapid stream of air, which also provided stirring. Tests showed that this effectively removed the radon. 'Blank' experiments were made to determine the loss of water by evaporation during this process (about 0·3 ml.), and in all irradiation experiments this amount of water was pipetted into the Petri dish to make good the loss.

IRRADIATION BY X-RAYS AND THE DOSAGE MEASUREMENTS

In practically all cases the solution of enzyme prepared for irradiation was divided into three portions, one of which was irradiated by α-rays, one by X-rays and the third used as a control. In this way the effectiveness of the two types of radiation could be directly compared. All X-ray doses were delivered by a continuously evacuated X-ray tube operating at 500 kV, 5 mA. The radiation had a half-value layer of 5·2 mm. copper and an estimated effective wave-length of 56 X.U. (Meredith & Stephenson 1943). The dosage rate at the position of the solution was determined by a '100 r. Victoreen Condenser Dosemeter', which had been calibrated at the N.P.L. For exposures of over 5 min. a calibrated 'Hammer' dosemeter was used as an integrating monitoring instrument.

THE EFFECT OF β-RAYS

In preliminary calculations, it was assumed that the effect of β-rays would be the same as that of X-rays, but it was felt advisable to check this experimentally. For this purpose a glass bulb about 1 cm. long and of wall thickness about 0·1 mm., containing radon, was suspended in the centre of 9·5 ml. of enzyme solution in a tube of about 2 cm. diameter. To ensure reasonable uniformity of irradiation, the solution was stirred by bubbling air through from below the bulb. The bulb wall thickness was sufficient to absorb all the α-particles and yet not to absorb much of the β-ray energy. The result of this experiment (as will be shown later) was to justify the assumption that X- and β-rays were comparable in their action.

EXPERIMENTS WITH AIR-FREE SOLUTIONS

Two identical glass bulbs of about 20 ml. capacity, at the end of 25 cm. long tubes, containing about 10 ml. of identical enzyme solutions ($3{\cdot}3 \times 10^{-5}$ g. enzyme/ml. solution) were suspended from a T-piece which, through a stopcock, was connected to a high-vacuum oil pump. The system between pump and stopcock was evacuated before the experiment started. The stopcock was opened for 15 sec. and the bulbs shaken. This

procedure was twice repeated. Then one bulb was fused off under vacuum at the middle of the stem, the other cut off and oxygen bubbled through whilst shaking. The two bulbs with their content were then irradiated with X-rays.

There was no difference in the degree of inactivation between the oxygenated and air-free enzyme solution.

Experiments with hydrogen peroxide

To decide whether hydrogen peroxide has an effect on carboxypeptidase, an amount of H_2O_2 was added to the enzyme, such that the final concentration of H_2O_2 was 0·1 M. There was no difference between the activity of the enzyme in the presence or the absence of H_2O_2.

The calculation of α- and β-ray doses

The α- and β-ray doses were assessed in 'energy units' (Gray, Mottram, Read & Spear 1940). The estimation of dose is less simple than that in the case of biological material immersed in radon solution, discussed by Gray & Read (1942), because the exposure is terminated by the addition of substrate before the active deposit laid down in solution has decayed and made its full contribution to the dose. The dose due to the α- and β-rays of all bodies, except the radon itself, must therefore be calculated separately allowing for growth and decay. This was done using accepted decay constants (Rutherford, Chadwick & Ellis 1930)—assuming the energies of the Ra, Ra A and Ra C′ α-particles and recoil atoms to be $5{\cdot}5_3$, $6{\cdot}0_7$ and $7{\cdot}8_2$ MeV respectively, and the mean energies of the Ra B and Ra C β-rays to be 0·23 and $0{\cdot}86_5$ MeV. The doses were then found from the formula (Gray & Read 1942)

$$\text{Dose} = 10^6\, E.N. \times \frac{4{\cdot}8 \times 10^{-10}}{W} \times \frac{n_{\text{air}}}{n_{\text{water}}}$$

$$= 1{\cdot}72 \times 10^{-8}\, E.N. \text{ energy units},$$

where N is the number of particles emitted per unit volume of solution by a given type of atom; E the energy in MeV of each of these particles; W the average energy in eV expended by fast electrons in producing a pair of ions in air ($W = 32{\cdot}5$) and n_{air} and n_{water} are, respectively, the number of electrons per unit volume of air at N.T.P. and per unit volume of water;

$$\frac{n_{\text{air}}}{n_{\text{water}}} = \frac{1}{860}.$$

There are two elements of uncertainty in these calculations. The first concerns the contribution from the glass splinters of the radon bulb, some of the radiations from which will be absorbed in the glass and therefore will not affect the main solution. It was decided that half the total energy of both the α- and β-particles emitted from the splinters should be considered as being absorbed by the solution. In the later experiments the correction was in any case small.

The second uncertainty arises from the fact that the radon is not completely removed as soon as the tube is opened; the amount in solution probably decreases roughly exponentially during the removal period. By taking samples of solutions during and after the radon removal period it was estimated that 1 min. could be taken as the 'average life' of

the radon in the solution, after the opening of the vessel; when calculating the dose 1 min. was, therefore, added to the main exposure and deducted from the time of the last stage of the irradiation process.

The following is a protocol of a typical experiment.

Protocol of a typical experiment

20. xi. 45. Enzyme concentration $= 2{\cdot}70 \times 10^{-5}$ g./ml.

Radon experiment

Radon bulb filled	14.11 hr.	Active deposit on glass due to 2 min. exposure to radon.
Radon bulb broken	14.13 hr.	Main exposure 14 min. + 1 min.
Vessel open for removal of radon	14.27 hr.	Main active deposit exposure 6 min. less 1 min. = 5 min.
Substrate added	14.33 hr.	Overall time 22 min.

Amount of radon used (average of three values) = 70 mC.

Volume of solution used = 22·1 ml.

'Energy units' delivered:

	from splinters*	from main exposure	from residual active deposit	totals
α-rays	658	17298	2334	20290
β-rays	20	51	69	140

Titration values

For irradiated solution 0·90 and 0·87; mean $= 0{\cdot}88_5$ ml. 0·02 N-NaOH

'Blank' 0·54 and 0·56; mean $= 0{\cdot}55_0$ ml. 0·02 N-NaOH

Difference $= \overline{0{\cdot}33_5}$ ml. 0·02 N-NaOH

For control solution 1·12 and 1·17; mean $= 1{\cdot}14_5$ ml. 0·02 N-NaOH

'Blank' 0·60 and 0·57; mean $= 0{\cdot}58_5$ ml. 0·02 N-NaOH

Difference $= \overline{0{\cdot}56_0}$ ml. 0·02 N-NaOH

Thus: $$\frac{\text{concentration after irradiation}}{\text{initial concentration}} = \frac{A}{A_0} = \frac{0{\cdot}33_5}{0{\cdot}56_0} = \underline{0{\cdot}59_8}$$

X-ray experiment

Dose delivered = 1292 r.

Titration values

For irradiated solution	$0{\cdot}93_0$ ml.	0·02 N-NaOH
'Blank'	$0{\cdot}58_5$ ml.	0·02 N-NaOH
Difference =	$\overline{0{\cdot}34_5}$ ml.	0·02 N-NaOH
For control solution	$1{\cdot}14_5$ ml.	0·02 N-NaOH
'Blank'	$0{\cdot}58_5$ ml.	0·02 N-NaOH
Difference =	$\overline{0{\cdot}56_0}$ ml.	0·02 N-NaOH

* Assuming that half the α- and β-rays irradiate the solution.

Therefore $$\frac{A}{A_0} = \frac{0{\cdot}34_5}{0{\cdot}56_0} = \underline{0{\cdot}615}$$

Assuming that for X-rays $A/A_0 = e^{-kd}$ (Dale *et al.* 1943) we find that 1367 r. is the dose of X-rays which would give the same inactivation of enzyme as was observed in the radon experiment, viz. $A/A_0 = 0{\cdot}59_8$.

Relative efficiency of α-rays and X-rays

20,290 energy units of α-rays + 140 energy units of β-rays are equivalent to 1367 r. of X-rays. Thus, assuming that X-rays and β-rays are equally efficient,

20,290 energy units of α-rays = 1367 − 140 = 1227 r. of X-rays.

Therefore $$\frac{\text{ionic efficiency of } \alpha\text{-rays}}{\text{ionic efficiency of X-rays}} = \frac{1{,}227}{20{,}290} = 0{\cdot}06_0.$$

It is evident, upon inspection of the protocol, that in this experiment only a fraction, 140/1367, or about 11%, of the observed inactivation of enzyme can be attributed to β-radiation, and therefore that a quite definite inactivation has been brought about by the α-radiation. Since, however, we wish to discover whether the efficiency of the α-radiation relative to X-radiation (in this experiment 6%) varies with enzyme concentration, and to consider the results more closely in certain other respects, it is necessary to assess the accuracy which could be assigned to the various estimates of X-ray and α-ray ionic yield. This will next be discussed.

The accuracy to be assigned to individual determinations of ionic yield

The primary experimental data consists of α-ray and X-ray dose estimations and of chemical titrations. The standard error in the measurement of X-ray dose should not exceed 2%. Sources of error in the estimation of α-ray doses have already been discussed. They introduce greater uncertainty than the possible errors in X-ray dosimetry, but, even so, may be neglected in almost all cases in comparison with the unavoidable errors in the estimation of enzyme activities. We, therefore, consider here only the accuracy of the chemical estimations.

Enzyme activity is taken to be proportional to the number of amide linkages of the substrate split, under precisely defined conditions, and is obtained as a difference between two formol titrations in ml. of 0·02 N-NaOH. It is assumed that experimental values are normally distributed about the mean and the accuracy of the mean is expressed in terms of its standard error.

From an examination of sixteen pairs of replica titrations it was concluded that the standard error of a titration was 0·024 ml. of a standard solution of 0·02 N-NaOH.

On five occasions, two samples of the same enzyme solution were separately digested and estimated. If it is assumed that the principal source of error in the estimation of enzyme activity is represented by the titration errors referred to above, so that a standard error may be calculated for each estimated enzyme activity, then the value of χ^2 may be calculated from the observed differences between the individual observations and their means. We find $\chi^2 = 5{\cdot}02$ for $n = 5$, corresponding to a probability $P = 0{\cdot}45$, which is satisfactory.

Accordingly, the standard error of all estimations of enzyme activity quoted in the paper has been calculated by assuming a standard error of 0·024 ml. in the individual titrations from which enzyme activities were estimated.

In the two cases (see below and p. 47) in which a linear relation has been postulated between certain functions of the dose, the enzyme concentration and the residual enzyme activity, the scatter of the points about the best straight line which could be drawn is greater than would be consistent with the postulated standard error for each determination of enzyme activity, and the scatter is of a kind which would not be much reduced by postulating a more complicated function than a linear relation. It is relevant that all the experimental observations which show a scatter greater than that to be expected were made with very dilute enzyme solutions (less than 2×10^{-5} g. enzyme/ml.), the behaviour of which has been discussed (p. 35). If observations with solutions of extreme dilution, i.e. less than $2{\cdot}2\times10^{-5}$ g./ml., are excluded the agreement between all estimates of the ionic yield made at the same or closely similar enzyme concentrations, even though separated in time by periods up to 2 years, are consistent with the estimated standard error of the determination of each enzyme activity.

Results

In table 2 will be found figures representing enzyme activities A_0 and A before and after irradiation by X-, β- and α-radiation. These figures are, in fact, the values in ml. of 0·02 N-NaOH used to titrate the amide linkages of the substrate split after digestion with enzyme. Other columns in the table give the dose of radiation delivered, the estimated inactivation dose D, the concentration of enzyme C, and the specific inactivation dose D/C.

In the case of the X-ray data the experiments have been grouped in such a way as to provide a smaller number of more accurate estimates of the specific inactivation dose, spaced at convenient intervals over the whole range of concentration investigated. In the case of the α-ray exposures the dose given is that due to the α-rays alone.

Characteristics of inactivation by X-rays

In confirmation of earlier experiments (Dale *et al.* 1943), the inactivation of enzyme over the range of inactivations studied is exponentially related to the dose, as shown in figure 3. The inactivation under any given experimental conditions may therefore be expressed quantitatively in terms of the 'inactivation dose' (D), which is the dose which would reduce the activity to $1/e$ of the initial value.

Data obtained in connexion with the present investigations covered an extremely wide range of enzyme concentrations, namely from 5×10^{-6} to 0·15 g. enzyme/ml. of solution,* and it is of interest to examine the influence of enzyme concentration on the inactivation dose.

As pointed out in earlier papers (Dale 1940, 1942; Dale *et al.* 1943) the inactivation dose for a pure enzyme solution should be proportional to the relative weights of enzyme and water in solution, provided that no appreciable proportion of enzyme is inactivated by direct ionization.

* Technical details of experiments with highest concentration of enzyme protein differed from the normal procedure and will be published elsewhere.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

TABLE 2. THE INACTIVATION OF CARBOXYPEPTIDASE BY X-RAYS, β-RAYS AND α-RAYS—EXPERIMENTAL DATA

A. *Inactivation by X-rays*

date	enzyme concentration C in g./ml. $C\times10^5$	dose in roentgens	activity of enzyme in equivalent ml. of 0·02 N-NaOH: initial A_0	activity of enzyme in equivalent ml. of 0·02 N-NaOH: final A	A/A_0	inactivation dose D	specific inactivation dose $D/C\times10^{-7}$: value derived from experiment	specific inactivation dose $D/C\times10^{-7}$: standard error	weighted mean values: enzyme concentration C in g./ml. $C\times10^5$	weighted mean values: specific inactivation dose $D/C\times10^{-7}$	weighted mean values: standard error
3. v. 45	$0{\cdot}45_5$	190	0·40	0·33	0·825	986	21·6	± 10·6			
12. vi. 45	$0{\cdot}46_6$	281	$0{\cdot}39_5$	0·32	0·810	1,334	28·5	± 9·3	$0{\cdot}58_2$	20·52	± 3·16
6. ix. 43*	$0{\cdot}61_1$	426	$0{\cdot}52_5$	$0{\cdot}36_5$	0·695	1,171	19·2	± 3·5			
29. vi. 45	$1{\cdot}45_5$	257	$0{\cdot}65_5$	$0{\cdot}64_5$	0·985						
,,	$1{\cdot}45_5$	565	$0{\cdot}65_5$†	$0{\cdot}32_5$	0·496						
,,	$1{\cdot}45_5$	950	$0{\cdot}65_5$	$0{\cdot}32_5$	0·496	1,450	10·0	± 1·1			
,,	$1{\cdot}45_5$	1,454	$0{\cdot}65_5$	$0{\cdot}26_5$	0·405						
,,	$1{\cdot}45_5$	2,200	$0{\cdot}65_5$	$0{\cdot}17_5$	0·267				$1{\cdot}53_5$	11·4	± 0·70
,,	$1{\cdot}45_5$	3,650	$0{\cdot}65_5$	0	0						
12. vi. 45	1·57	400	$0{\cdot}91_5$	$0{\cdot}67_5$	0·737						
,,	1·57	780	$0{\cdot}91_5$†	$0{\cdot}64_5$	0·705	1,902	12·1	± 2·4			
,,	1·57	1,000	$0{\cdot}91_5$	0·51	0·557						
15. vi. 43*	1·82	2,000	0·48	0·21	0·438	2,411	13·28	± 1·51			
5. vii. 43*	1·82	4,150	0·43	0·07	0·163	2,287	12·55	± 2·18	1·82	13·15	± 0·90
9. vii. 43*	1·82	4,000	0·73	0·14	0·192	2,423	13·30	± 1·29			
20. xi. 45	2·70	1,292	0·56	$0{\cdot}34_5$	0·615	2,668	9·88	± 1·59			
23. iv. 45	2·99	1,250	0·80	0·38	0·475	1,679	5·61	± 0·58	2·98	6·05	± 0·48
16. i. 45	3·05	4,010	0·56	0·06	0·107	1,795	5·90	± 1·00			
12. vi. 45	4·69	2,000	$1{\cdot}26_5$	0·85	0·672	5,029	10·73	± 0·68	4·69	10·73	± 0·68
,,	46·9	7,800	$1{\cdot}34_5$	1·02	0·757	28,200	6·01	± 0·46	46·9	$6{\cdot}01_5$	± 0·46
26. i. 45	564	55,000	$1{\cdot}45_5$	1·21	0·832	298,300	5·30	± 0·74	564	5·61	± 0·54
29. iii. 45	564	65,000	$1{\cdot}51_5$	1·25	0·825	338,100	6·00	± 0·78			
14. ii. 45	6,080	500,000	0·91	0·75	0·824	2,588,000	4·26	± 0·92	6,080	4·26	± 0·92
26. ii. 45	15,550	1,205,000	$0{\cdot}93_5$	$0{\cdot}81_5$	0·874	8,855,000	5·69	± 1·63	15,550	5·69	± 1·63
,,	15,550	2,133,000	$0{\cdot}93_5$	$0{\cdot}72_5$	0·774	8,339,000	5·36	± 0·89	15,550	5·36	± 0·89

B. *Inactivation by β-rays*

date	enzyme concentration C in g./ml. $C\times10^5$	dose in roentgens	activity of enzyme in equivalent ml. of 0·02 N-NaOH: initial A_0	activity of enzyme in equivalent ml. of 0·02 N-NaOH: final A	A/A_0	inactivation dose D	specific inactivation dose $D/C\times10^{-7}$: value derived from experiment	specific inactivation dose $D/C\times10^{-7}$: standard error	weighted mean values: enzyme concentration C in g./ml. $C\times10^5$	weighted mean values: specific inactivation dose $D/C\times10^{-7}$	weighted mean values: standard error
23. i. 45	2·98	1,230	0·80	0·51	0·637	2,733	9·15	± 1·60	2·98	9·15	± 1·60

C. *Inactivation by α-rays*

date	enzyme concentration C in g./ml. $C\times10^5$	dose in energy units	activity of enzyme in equivalent ml. of 0·02 N-NaOH: initial A_0	activity of enzyme in equivalent ml. of 0·02 N-NaOH: final A	A/A_0	equivalent X-ray dose: associated observations‡	equivalent X-ray dose: smooth curve	β-ray dose	equivalent (X-ray—β-ray) dose: associated observations	equivalent (X-ray—β-ray) dose: smooth curve	ratio of α-ray to X-ray efficiency η_x^α: associated observations	ratio of α-ray to X-ray efficiency η_x^α: smooth curve
3. v. 45	$0{\cdot}45_5$	3,815	0·400	0·250	0·625	466	539	50	416	489	$10{\cdot}9\pm7{\cdot}3$	$12{\cdot}8\pm4{\cdot}7$
6. ix. 43	0·61	9,965	0·525	0·295	0·560	675	713	322	353	391	$3{\cdot}5\pm1{\cdot}4$	$3{\cdot}9\pm0{\cdot}8$
20. xi. 45	2·70	20,290	0·560	0·335	0·598	1,367	1,225	140	1,227	1,085	$6{\cdot}0\pm1{\cdot}5$	$5{\cdot}3\pm0{\cdot}9$
,,	2·70	13,600	0·560	0·390	0·697	964	861	96	868	765	$6{\cdot}4\pm1{\cdot}9$	$5{\cdot}6\pm1{\cdot}3$
16. i. 45	3·05	104,800	0·560	0·040	0·071	4,710	6,830	2,373	2,337	4,457	$2{\cdot}2\pm1{\cdot}3$	$4{\cdot}3\pm1{\cdot}5$
19. i. 45	90·3	133,300	0·895	0·780	0·871	—	7,010	2,845	—	4,165	—	$3{\cdot}1\pm1{\cdot}9$
26. i. 45	564	880,700	1·455	1·040	0·714	100,800	105,000	24,690	76,110	80,310	$8{\cdot}6\pm2{\cdot}0$	$9{\cdot}1\pm1{\cdot}3$
29. iii. 45	564	1,165,000	1·515	0·980	0·650	146,500	134,300	29,180	117,320	105,120	$10{\cdot}0\pm2{\cdot}0$	$9{\cdot}0\pm1{\cdot}0$

* In these experiments the substrate was edestin. In all other experiments it was chloracetyltyrosine.
† Initial value measured once only.
‡ Values derived from X-ray exposures made at the time of the α-ray exposures.

We should, therefore, expect the ratio of inactivation dose to concentration, which we have defined as the specific inactivation dose (D/C), to be independent of concentration over a wide range. The experimental data are plotted in figure 4. There is quite evidently an increase in the specific inactivation dose at very low enzyme concentrations. On the other hand, for all the enzyme concentrations greater than about $2{\cdot}5 \times 10^{-5}$ the values of the specific inactivation dose (D/C) show no systematic departure from the mean value*

$$D/C = (6{\cdot}36 \pm 0{\cdot}24) \times 10^7 \text{ r./g./ml.}$$

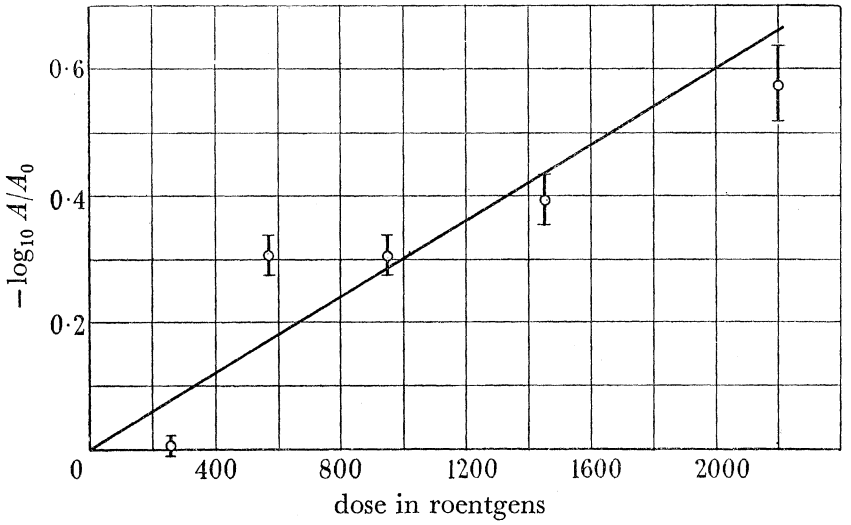


FIGURE 3. The inactivation of carboxypeptidase by X-rays. The relation between the degree of inactivation and dose. The straight line gives the best fit as computed by the method of least squares. The standard deviations are derived on the assumption of a titration error only of 0·024 ml. (cf. p. 44).

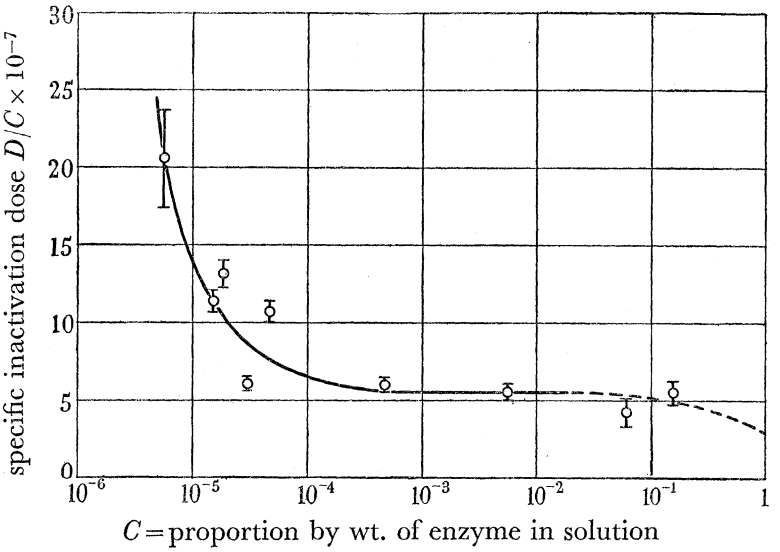


FIGURE 4. The inactivation of carboxypeptidase by X-radiation. The relation between the specific inactivation dose and the concentration of the enzyme solution.

* If all observations are included, the scatter about the mean is much greater than would be expected ($\chi^2 = 57$, $n = 9$, $P < 0{\cdot}001$) but one point alone ($D/C = 10{\cdot}7 \pm 0{\cdot}7$ at $C = 4{\cdot}69 \times 10^{-5}$ g./ml.) contributes 42 to χ^2. If this point is disregarded the remaining nine observations give $\chi^2 = 16$ for $n = 8$. The probability of obtaining this result is $P = 0{\cdot}05$ indicating that the scatter of these nine observations is not to be regarded as abnormal.

Over the whole range of concentrations the observations may be fitted by an equation of the form $(D/C) = P + Q/C$, where P and Q are constants.

If the constants are evaluated from the experimental observations (figure 5) by the method of least squares we find

$$P = (5{\cdot}2 \pm 0{\cdot}8) \times 10^7, \quad Q = (8{\cdot}9 \pm 2{\cdot}6) \times 10^2,$$

so that the best estimate of the relation between inactivation dose and concentration for X-rays is

$$D = 5{\cdot}2 \times 10^7\, C + 8{\cdot}9 \times 10^2.$$

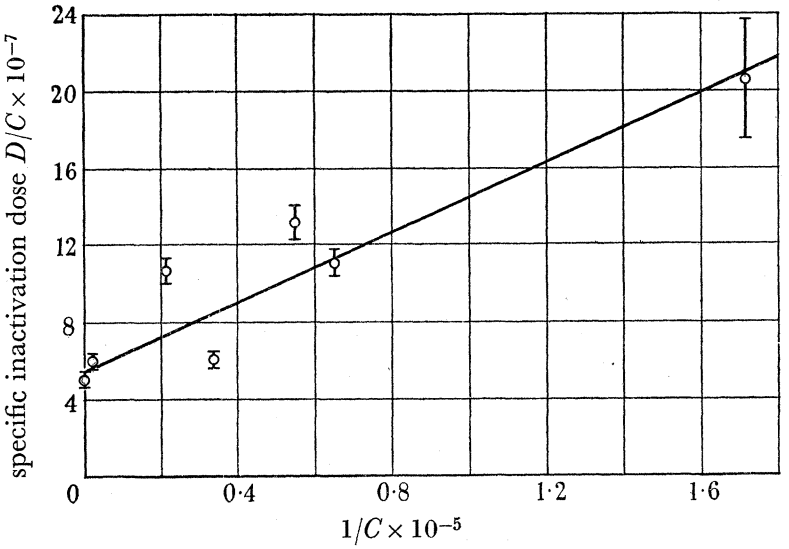


FIGURE 5. The inactivation of carboxypeptidase by X-radiation. The relation between specific inactivation dose and the reciprocal of the enzyme concentration.

This is the expected form of the relation between dose and concentration if the intermediate product formed by the radiation consists of radicals capable of recombination or if the enzyme is in competition with another solute as, for example, dissolved oxygen. Variations in the concentration of dissolved oxygen, or any other competitor, could obviously be an additional cause of scatter in the observations at extreme dilutions.

The specific inactivation dose for β-radiation

When enzyme is exposed to the α-radiation from radon, Ra A and Ra C′ some small part of the observed inactivation is due to the β-rays from Ra B and Ra C. These two groups of β-particles, which are emitted in equal numbers, have mean energies of 0·23 and 0·86 MeV respectively, and are, therefore, considerably more energetic than the recoil electrons generated by 500 kV X-rays, which were adopted as the standard of comparison. In linear ion density, however, the two radiations do not differ greatly—the mean value for the Ra B and Ra C β-rays is about 10 ions/μ and that for 500 kV X-rays about 34 ions/μ. The difference between these two is very small compared with that between either of them and the ion density of α-radiation, which is about 3500 ions/μ. If anything, the β-radiation might have been expected to be slightly more efficient than the X-radiation.

The experimental values of the specific inactivation dose, when a solution containing $2{\cdot}98 \times 10^{-5}$ g. of enzyme/ml. is exposed to β-radiation (table 2), is

$$(D/C = 9{\cdot}15 \pm 1{\cdot}6) \times 10^7 \text{ r./g./ml.}$$

At this enzyme concentration, the X-ray specific inactivation dose is

$$(8{\cdot}5 \pm 0{\cdot}8) \times 10^7 \text{ r./g./ml.}$$

The β-radiation thus appears slightly less efficient than 500 kV X-radiation but the difference is only 0·3 times the standard error of the difference and is not significant.

Characteristics of inactivation by α-radiation

In almost every case the inactivation of enzyme brought about by exposure to radon was compared directly with that brought about by exposing a sample of the same enzyme solution to a much smaller dose of X-radiation. The doses were chosen with the aim of producing about 50 % inactivation in each case and the two irradiations were then carried out practically simultaneously. The contribution from the β-rays was allowed for by assuming that β-radiation has the same efficiency as X-radiation. The value of the ratio of the X-ray and α-ray inactivation doses, which we referred to as the efficiency of α-radiation relative to X-radiation (η_x^α) was calculated in the manner set out in the protocol example (p. 42).

When, however, all the X-ray results were analysed statistically (cf. p. 44), it became apparent that individual determinations, separated by a long period of time, of the degree of inactivation brought about by a given dose, did not differ from the mean to an appreciably greater extent than determinations made on the same day. It is obviously more accurate to compare the α-ray inactivation at any given concentration with the value calculated from the relation $D = 5{\cdot}2 \times 10^7\, C + 8{\cdot}9 \times 10^2$ which has been established for X-rays, rather than with the individual X-ray observations made on the same occasion as the α-ray exposure. Accordingly, the values of η_x^α were recalculated in this way. Both sets of results are given in table 2. The second set have the lower standard deviations and are to be preferred. Grouped values are given in table 3.

TABLE 3. EFFICIENCY OF α-RADIATION RELATIVE TO X-RADIATION, AND THE ABSOLUTE α-RAY IONIC YIELD, AS A FUNCTION OF ENZYME CONCENTRATION

$C \times 10^5$	0·58	2·77	90·3	564
$\eta_x^\alpha \times 10^2$	$5{\cdot}8 \pm 1{\cdot}0$	$5{\cdot}2 \pm 0{\cdot}7$	$3{\cdot}1 \pm 1{\cdot}9$	$9{\cdot}0 \pm 0{\cdot}8$
$(M/N)_\alpha \times 10^3$	$2{\cdot}7 \pm 0{\cdot}4$	$5{\cdot}9 \pm 0{\cdot}8$	$5{\cdot}7 \pm 3{\cdot}5$	$16{\cdot}6 \pm 1{\cdot}5$

The dependence of η_x^α on enzyme concentration

The efficiency of α-radiation relative to X-radiation appears to be about 5 % at the lowest enzyme concentrations examined, and to increase slowly with enzyme concentration (figure 6*a*). At 6×10^{-3} g./ml., the highest concentration for which α-ray data were obtained, the relative efficiency of α-radiation was found to be about 9 %.

The possibility that η_x^α might be proportional to enzyme concentration was also examined statistically, using the experimental values of the relative ionic yield listed in table 2. On the suggested hypothesis we obtain $\chi^2 = 94$ for 7 degrees of freedom, the probability of which is exceedingly small. The experimental results may therefore be taken to establish beyond doubt that the relative α-ray ionic yield is not proportional to enzyme concentration. The interpretation of this result will be considered later (p. 57).

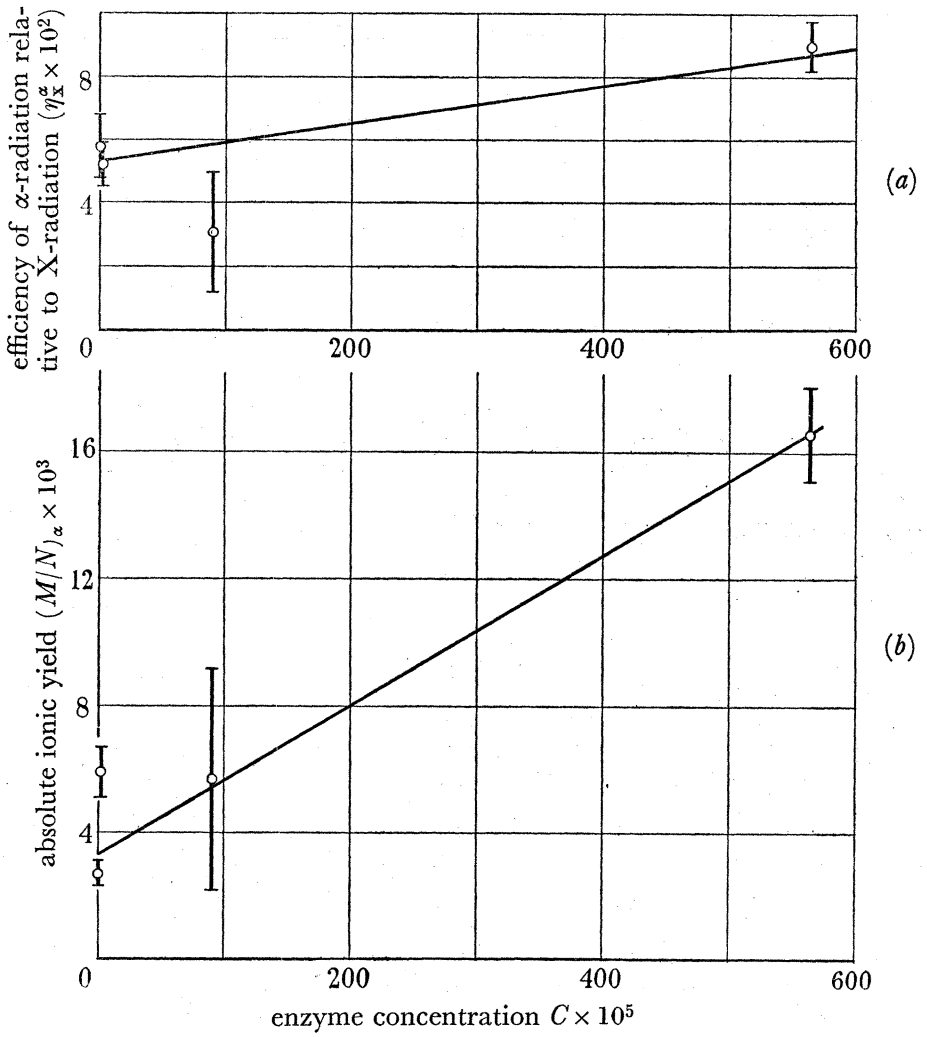


FIGURE 6. (*a*) The inactivation of carboxypeptidase by X- and α-radiation. Ratio of α- to X-ray ionic yield as a function of enzyme concentration. (*b*) The inactivation of carboxypeptidase by α-radiation. The absolute ionic yield as a function of enzyme concentration.

The absolute ionic yield of X-radiation and α-radiation

The molecular weight of carboxypeptidase was determined by one of us (W. M. D.) in collaboration with Dr R. A. Kekwick and Dr B. R. Record, of the Lister Institute, by means of the ultracentrifuge.

A 1% enzyme solution ($c = 10^{-2}$) was used in 0·1 M-NaCl at pH ~9·0. The solution showed a single sedimenting boundary with a sedimentation constant $s_{20} = 3{\cdot}6 \times 10^{-13}$ cm./sec., which places the enzyme in the class of proteins of molecular weight 35,000. The diffusion constant, as judged by the spread of the boundary, was estimated at $D_{20} = 15 \times 10^{-7}$ cm.^{2}/sec., which is appreciably greater than the normal for proteins of the 35,000 molecular weight class, and suggests a certain amount of polydispersity in the specimen examined. This was possibly due to the enzyme being, for a short period, in a frozen state whilst in transit.

Since the molecular weight was determined at an enzyme concentration well within the range for which the X-ray specific inactivation dose was observed to be constant, we may calculate the ionic yield as follows.

The specific inactivation dose $D/C = 5{\cdot}2 \times 10^7$ r./g./ml. for 500 kV X-rays. This dose produces

$$5{\cdot}2 \times 10^7 \times 1{\cdot}79 \times 10^{12}\left(\frac{W_{\text{air}}}{W_{\text{water}}}\right) = 9{\cdot}3 \times 10^{19}\left(\frac{W_{\text{air}}}{W_{\text{water}}}\right)\text{ions/g. of water,}$$

where W_{air} and W_{water} are the energies required to produce a pair of ions in air and water respectively.

$$\text{1 g. carboxypeptidase contains } \frac{6{\cdot}03 \times 10^{23}}{3{\cdot}5 \times 10^4} = 1{\cdot}725 \times 10^{19} \text{ molecules of enzyme,}$$

$$\text{therefore} \qquad \text{ionic yield}\left(\frac{M}{N}\right)_X = \frac{1{\cdot}725 \times 10^{19}}{9{\cdot}3 \times 10^{19}}\left(\frac{W_{\text{water}}}{W_{\text{air}}}\right) = 0{\cdot}18_5\left(\frac{W_{\text{water}}}{W_{\text{air}}}\right),$$

or, since $\left(\frac{W_{\text{water}}}{W_{\text{air}}}\right)$ is not known precisely but is of the order of unity

$$(M/N)_X \simeq 0{\cdot}18.$$

Thus, one molecule of carboxypeptidase is indirectly inactivated for every six ion pairs formed in the water by X-radiation.

Since it must require at least one ion cluster averaging three ion pairs to cause inactivation by the direct ionization of the enzyme molecule, the ionic yield by the indirect process cannot, in the case of X-radiation, be less than half that by the direct process. For this reason, the departure of the specific inactivation dose from constancy at high concentrations cannot be greater than is indicated by the broken line in figure 4.

Since the mean value of $\eta_x^\alpha = 0{\cdot}065$ the absolute ionic yield for indirect inactivation of carboxypeptidase by α-radiation is approximately

$$(M/N)_\alpha \sim 0{\cdot}06 \times 0{\cdot}18 \sim 0{\cdot}01.$$

Taking into account the proportion of occasions on which the α-particle will leave more than one ion cluster in a molecule of the size of carboxypeptidase, the α-ray inactivation dose for the direct ionization of the enzyme is $6{\cdot}6 \times 10^7$ energy units (Lea 1946, figure 9) from which it may be estimated that having regard to the accuracy of our individual experimental determinations of inactivation dose, we might expect in the case of α-radiation to detect the influence of the direct ionization of the enzyme at concentrations above 3% ($C = 3 \times 10^{-2}$). The highest concentration studied, however, was only one-sixth of this, namely $C = 5{\cdot}64 \times 10^{-3}$.* If more than one ion cluster is required to inactivate the enzyme molecule the direct effect might only be noticeable at still higher concentrations. Table 3 gives the experimental values for the absolute α-ray ionic yield as a function of concentration (cf. also figure 6*b*). In this case, the dependence on concentration is undoubtedly statistically significant.

On statistical grounds, the hypothesis that $(M/N)_\alpha$ is directly proportional to concentration is so improbable that it may be considered incompatible with our observations.

* The viscosity of solutions of higher concentration and a tendency to recrystallization is incompatible with the technique of irradiation using radon by the method already described.

Summary of results

The results may be summarized as follows:

(1) *Inactivation by* 500 kV *X-rays and β-rays*

The present investigation has confirmed and extended previous observations by showing that the inactivation of pure carboxypeptidase in aqueous solution is exponentially related to the dose, thus, $A/A_0 = e^{-kd}$, and that the reciprocal of the exponent k, which is referred to as the inactivation dose D, is proportional to enzyme concentration over a wide range of concentrations, showing that up to the highest concentrations so far studied ($1{\cdot}55 \times 10^{-1}$ g. protein/ml.) the inactivation is in the main brought about by interaction with a labile product of the ionization of water. Over the whole range of enzyme concentrations studied, namely, from $0{\cdot}45 \times 10^{-5}$ to $1{\cdot}55 \times 10^{-1}$ g./ml., the inactivation dose is related to the concentration by the formula $D = 5{\cdot}2 \times 10^7 C + 8{\cdot}9 \times 10^2$. The estimated standard errors of the constants are $0{\cdot}8 \times 10^7$ and $2{\cdot}6 \times 10^2$ respectively. Over the restricted range of enzyme concentrations, $C = 2{\cdot}5 \times 10^{-4}$ to $C = 1{\cdot}5 \times 10^{-1}$, the specific inactivation dose D/C is approximately constant and equal to $(6{\cdot}4 \pm 0{\cdot}2_5) \times 10^7$ r./g./ml. Since the molecular weight of the enzyme is about 35,000 the absolute X-ray ionic yield is also constant over this range of concentrations and is given by $\left(\frac{M}{N}\right)_X = [0{\cdot}18 \pm 0{\cdot}03]\left(\frac{W_{\text{water}}}{W_{\text{air}}}\right)$, where $\left(\frac{W_{\text{water}}}{W_{\text{air}}}\right)$ is of the order of unity.

As indicated by the formula relating D and C, the ionic yield decreases with decreasing enzyme concentration. This may be due to the influence of dilution on the character of the enzyme molecule, to competition with another solute for the labile product formed in the water by the X-radiation, or to the spontaneous disappearance of this product.

Absence of dissolved oxygen does not affect the degree of inactivation of a 3×10^{-5} g./ml. enzyme solution by X-radiation. At the one concentration studied, the inactivation by β-radiation is indistinguishable from that produced by the same dose of 500 kV X-radiation.

The enzyme is not inactivated by H_2O_2.

(2) *Inactivation by α-radiation*

A technique for the use of radon as a source of radiation has been used, which reduces the influence of β-rays to such an extent that the inactivation observed is predominantly due to α-radiation. It has thus been possible to show that carboxypeptidase is inactivated by α-radiation through the intermediary of the labile product formed by the ionization of the water. The ionic yield, however, is low, the average value $(M/N)_\alpha$ being about 0·01 over the range of enzyme concentrations examined. The ionic yield appears to increase with enzyme concentration at concentrations for which the X-ray ionic yield is constant (table 3). This cannot be ascribed to inactivation by direct ionization of the enzyme molecule.

Discussion

The particularly striking feature of these results is the low value of the α-particle ionic yield compared with the X-ray ionic yield, and it is of interest to enquire whether this difference in ionic yield for two radiations of very different linear ion densities throws any

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

light on the mechanism by which the inactivation is brought about. Any proposals for this mechanism must also be in keeping with the observed differences between the effects of X-rays and α-rays on water; i.e. the high efficiency of α-rays in producing gas from water compared with the low, or even negligible efficiency of X-rays.

It will be convenient to treat the discussion under the following headings:

(1) Ionization by X- and α-rays, including (1*a*) recombination of the ions formed by X- and α-radiation, and (1*b*) possible conversion of ions to radicals.

(2) Correlation of differences between X- and α-ray ionization with the effects of irradiating (2*a*) pure water, (2*b*) solutes dissolved in air-free water, (2*c*) oxygenated water and (2*d*) carboxypeptidase dissolved in oxygenated water.

(1) *Ionization by X- and α-rays*

The rate of loss of energy by a charged particle passing through a material medium is a function of its speed and proportional to the square of its charge. Thus, when comparing the effects of α-particles and electrons of the same speed, the relative frequency of occurrence of particular types of ionization and excitation will be the same in the two cases, but the doubly charged α-particle will produce just four times as many ionizations and excitations of each type per cm. of path. A 5 MeV α-particle, however, has only the speed of a 0·7 kV electron. In the experiments just described the electrons were generated by 500 kV X-rays and had a mean initial energy of about 60 kV. We are, therefore, concerned with the effects produced by particles of very dissimilar speed. When, as in the case of 60 kV electrons, the particle speed is large compared with that of the orbital electrons of the molecule ionized, the distribution of the total energy loss among the various types of ionization and excitation is practically independent of the speed. In the case of the α-particle which has a speed comparable with that of the *K* electrons of oxygen, the manner in which the total energy loss is distributed cannot confidently be assumed to be the same as for faster particles. The number of ions formed per unit total loss of energy is, however, known to be of the same order for α-particles and electrons in a number of gases (Gray 1944). Since we are here concerned to find a reason for a twenty-fold difference in ionic efficiency between the two radiations, it is probably safe to neglect all differences between α-ray and electron ionization other than the most striking one of the spatial distribution of the ions. The positive ions primarily produced are, in each case, formed along the geometrical path of the ionizing particle, but the mean linear ion densities differ by about a factor of 100, the distance between successive primary positive ions being only $8 \times 10^{-4} \mu$ in water along an α-particle track compared with about $9 \times 10^{-2} \mu$ along the track of a 60 kV electron* (Lea 1946). The ejected electrons have on the average sufficient energy to form two more pairs of ions before coming to rest by attachment to a water molecule to form a negative ion. The ionization thus occurs in clusters of one, two, three or more pairs of ions; from experimental observations in hydrogen (inferred from cloud chamber photographs of α-particle tracks (Klemperer 1927)) and hexane (experimental observations by W. Mies, quoted by Jaffé 1913) it would seem (Lea 1946) that the dimensions of the clusters in water are probably of the order $2 \times 10^{-2} \mu$. The clusters are thus relatively widely spaced along a β-particle track, barely discrete along a 60 kV recoil electron track and

* The mean initial energy of a recoil electron produced by X-radiation from a tube operated at 500 kV.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

form an almost continuous cylinder of much denser ionization along an α-particle track. It should be noted that some of the ejected electrons have sufficient energy to produce a large number of ionizations on their own account. These electrons (δ-rays), which have an important role in our interpretation of the chemical effects of α-radiation, have all speeds up to twice that of the primary particle. The energy spectrum of the δ-rays has been investigated experimentally by Alper (1932), and computed by Lea (1946). Slow δ-rays are much more abundant than fast. A small proportion of them will, nevertheless, be sufficiently energetic to travel well clear of the primary column. Since the δ-rays are slow electrons their ion density will be intermediate between that of a fast electron and that of an α-particle.

In the absence of an electric field the ions within the column rapidly recombine in hexane. It is not known whether any permanent chemical change results.

(1*a*) *Recombination of the ions formed by X- and α-radiation*

The recombination process has been treated theoretically in some detail by Jaffé (1913), and by Kara-Michailova & Lea (1940). It is evident that during the time that the ions are recombining the radius of the column will be increasing on account of diffusion. If, within a column of initial radius b there are N_0 ions of each sign randomly distributed, then the expression deduced by Jaffé for the number N of ions remaining uncombined t seconds later is

$$N_0/N = 1 + \frac{\alpha N_0}{8\pi D} \log_e \left(\frac{b^2 + 4Dt}{b^2} \right),$$

where α = coefficient of recombination, and D = diffusion coefficient.

During this time the radius of the column has increased from b to $b' = \sqrt{(b^2 + 4Dt)}$. From this formula it appears that the time required for half the ions to recombine is less than 2×10^{-6} sec. for 60 kV electron ionization and less than 10^{-9} sec. for α-particle ionization.

(1*b*) *Possible conversion of ions to radicals*

In order to account for the effects of ionizing radiations on dilute aqueous solutions, Weiss (1944) has postulated that as a result of the act of ionization hydrogen and hydroxyl ions become converted into the corresponding radicals. The formation of radicals may be represented thus:

$$H_2O^+ \longrightarrow H^+ + OH, \quad H_2O^- \longrightarrow H + OH^-,$$

and it is supposed that the observed chemical reactions result from interaction between solute and either hydroxyl radical or hydrogen atom or both. Weiss assumes that in the absence of solute the hydrogen atoms and hydroxyl radicals recombine to form water.* Lea has pointed out that Jaffé's formula for ion recombination applies equally to recombination between the hydroxyl radicals and hydrogen atoms. The reaction between radicals and solute is therefore considered as in competition with the recombination of radicals, which is taking place within an expanding column in which the initial concentration of radicals is high. The curves given by Lea (1946, figure 4, p. 58) predict the manner in which the ionic yield for a given solute would depend on the linear ion density of the

* See, however, footnote, p. 55.

radiation used. As would be expected, the curves show that the concentration of solute at which the ionic yield commences to fall owing to loss of radicals by recombination within the column is about 100 times greater for α-particles than for electrons, corresponding to the difference in ion density of these two radiations.

While the positive and negative ions (and hence the radicals, if dissociation takes place sufficiently rapidly) may with fair approximation be considered randomly distributed within an electron column, this is far from true of the initial distribution of ions within an α-ray column. Primary positive ions lie on a straight line approximately $8 \times 10^{-4} \mu$ apart and are surrounded by a sheath of negative ions. It would, therefore, be more appropriate to consider the positive and negative columns separately in the case of α-radiation. For the purpose of calculation, we may in any given case assume the positive ions to be randomly distributed within a column having a radius equal to the mean separation of the ions. This picture is, however, somewhat modified by the δ-rays. These may conveniently be divided into three groups—δ-rays having (*a*) energies less than 100 eV, (*b*) energies between 100 and 1000 eV, and (*c*) energies in excess of 1000 eV. Those of group (*a*) have only enough energy to produce a few pairs of ions and their positive ions will be formed within the main negative sheath. Those of group (*b*) will produce ionization beyond the negative sheath and with an ion density of the same order as that for the α-ray, whilst the range of those of group (*c*) will be even greater and its ion density smaller. The initial distribution of radicals formed by a 60 kV electron, a 1 kV electron and an α-particle are given in table 4 together with approximate data for the expansion of the columns, derived by applying the Jaffé formula.

TABLE 4

	α-particle		1 kV electron		60 kV electron	
	H_2O^+ or OH*	H_2O^- or H	H_2O^+ or OH	H_2O^- or H	H_2O^+ or OH	H_2O^- or H
mean separation of primary positive ions (μ)	8×10^{-4}	—	5×10^{-3}	—	9×10^{-2}	—
radius of column (μ)	8×10^{-4}	$1{\cdot}5 \times 10^{-2}$	5×10^{-3}	$1{\cdot}5 \times 10^{-2}$	$1{\cdot}5 \times 10^{-2}$	
initial concentration of radicals (M)	1·0	$8{\cdot}7 \times 10^{-3}$	$1{\cdot}2 \times 10^{-2}$	$1{\cdot}35 \times 10^{-3}$	$7{\cdot}3 \times 10^{-5}$	
interval after which 50 % of radicals will have made one collision with another radical (sec.)	10^{-11}	10^{-9}	7×10^{-10}	6×10^{-9}	$1{\cdot}6 \times 10^{-6}$	
expansion of column radius during this interval b'/b	1·06	1·02	1·11		7·6	
concentration of radicals at the end of the interval (M)	0·9	$8{\cdot}4 \times 10^{-3}$	$1{\cdot}0 \times 10^{-2}$	$1{\cdot}1 \times 10^{-3}$	$1{\cdot}3 \times 10^{-6}$	

The data given in the table are calculated from the Jaffé formula, using the same values of the constants as used by Lea (1946), namely $D = 2 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ and $\alpha = 4 \times 10^{-1}$. Column radii are given in microns.

* Primary positive ions only are considered in the case of α-radiation. In the case of 1 kV electrons the positive column radius is large enough to include secondary positive ions.

It is to be noted that the time which elapses before chemical reaction between radicals takes place may be very much greater than the intervals shown in the fourth line of the table, since these intervals are only those required for half the radicals to make a single

collision with another radical. Consequently, as far as chemical reaction is concerned, the figures for column expansion are lower limits and those for radical concentration after 50% interaction are upper limits.

The figures in the table, nevertheless, probably give a correct general picture, and will serve as a basis for the discussion of the differences between one ionizing radiation and another. They appear to throw some light on the hitherto rather puzzling and conflicting experimental results concerning the decomposition of pure water. Lea has recently arrived at essentially the same conclusion.*

(2) *Consequences of the differences between X- and α-ray ionization*

(2 *a*) *Pure water exposed to X- and α-radiation*

There is no general agreement as to whether pure water is or is not decomposed by X-radiation. It is probably safe to conclude that if decomposition does take place it is with low ionic efficiency. We may infer from this that in the homogeneous liquid phase $H+OH \rightarrow H_2O$ is probably more rapid than any alternative reaction between these radicals, so that the majority of the radicals randomly distributed within a fast electron column probably recombine to form water.

It appears, on the other hand, to be established experimentally beyond doubt that water is decomposed by α-radiation. Duane & Scheuer (1913) found that when the α-ray dose was small the gas evolved was mainly hydrogen and an equivalent amount of hydrogen peroxide was formed in solution. With larger doses, hydrogen and oxygen are evolved in equivalent proportions, the ionic yield being of the order of unity. This suggests that the primary decomposition is into hydrogen peroxide and hydrogen. We suggest that this decomposition of water by α-radiation is to be correlated with the high concentrations of hydroxyl radicals which form the inner column along the track of an α-particle and the tracks of δ-rays of energies less than 1000 eV.

It seems not unreasonable to suppose that these radicals are formed with sufficient activation energy to combine to form hydrogen peroxide before a reacting collision takes place between a hydroxyl radical and a hydrogen atom, present in the surrounding sheath at much lower concentration (350 times less in the case of the main column). Thus we should expect that the primary α-ray ionization and that produced by electrons with energies between 100 and 1000 eV will have a high ionic efficiency in decomposing water. For δ-rays with energies below 100 eV the efficiency will be low because the radicals will

* At the Radiobiological Conference, held under the auspices of the British Institute of Radiology in May 1946, Lea read a paper entitled 'The Action of Radiations on Dilute Aqueous Solutions: the Spatial Distribution of H and OH', in which he distinguished between the positive and negative ion columns and gave approximate data for their rate of expansion. The paper has since been published (Lea 1947). In a private communication, received recently, Lea expressed the opinion, which we had reached independently and elaborate in the discussion which follows, that a consideration of the existence of the inner column of positive ions, giving rise to extremely high localized concentrations of hydroxyl radicals, is capable of explaining a number of the points of difference between the radiochemistry of α-radiation and X-radiation. Lea's quantitative treatment differs in some respects from ours, notably in that he considers the central column to consist of the primary positive ions together with the positive ions formed by secondary electrons having less than 100 V of energy. The radius of the positive column is taken as 0·003 μ (instead of 0·0008 μ as in table 4) so as to include these secondary positive ions. This treatment is possibly preferable to ours as a basis for a theoretical evaluation of α-ray ionic efficiency.

be formed within the negative sheath and therefore will not be well disposed to provide collisions between pairs of hydroxyl radicals.* The ionic efficiency with respect to peroxide production would be expected to fall off rapidly when the energy appreciably exceeds 1000 eV, since in this range of energies the ion density is falling rapidly and there will no longer be produced a high concentration of hydroxyl radicals. An α-particle produces few δ-rays of energy greater than 1 keV. The primary ionization produced by an α-particle plus that associated with δ-rays of energy between 100 and 1000 eV therefore accounts for a large part of the total ionization. The overall efficiency of α-radiation in decomposing water may thus be expected to be high, which is in keeping with the experimental results.

On the other hand, the efficiency of fast electrons will be low, since the only part of their ionization which has been postulated to have high ionic efficiency is that associated with secondary electrons of between 100 and 1000 eV. This fraction will vary with electron energy but for a 60 kV electron has been estimated at about 10%. The recoil protons generated by neutrons of energy up to about 4 MeV have ion densities comparable with that of 1 kV electrons and may therefore be expected to show a high ionic efficiency in decomposing water. Neutrons of greater energy would be expected to be less efficient.

(2*b*) *Solutes dissolved in air-free water*

We are thus led to expect that the variation of ionic yield with the concentration will depend not only on the ion density of the radiation employed but also on whether the reaction with the solute is such that it would undergo irreversible change by reaction with hydroxyl radicals or hydrogen atoms. A solute which reacts with hydroxyl radicals will clearly have to be present at a very high concentration indeed in order that any reaction may be observed when the solution is exposed to α-radiation. On the other hand, in the case of a solute which reacts with hydrogen atoms in a manner which cannot be reversed by subsequent reaction with hydrogen peroxide, a good ionic yield with α-radiation might be obtained at a concentration which is only about 100 times that at which good yields are obtained with X-radiation. It is even conceivable that α-radiation might be more effective than X-radiation in bringing about reactions of this type.†

(2*c*) *Oxygenated water exposed to X- and α-radiation*

Oxygen is presumably a solute which reacts with hydrogen atoms. The molarity of dissolved oxygen in equilibrium with air at atmospheric pressure is 3×10^{-4} M. This is some four times as great as the estimated initial concentration of hydrogen atoms within the 60 kV electron column and 2000 times greater than the estimated *maximum* concentration of radicals after 50% recombination has taken place.

It is therefore understandable that oxygen should be efficiently reduced to hydrogen peroxide by X-radiation even, as was found by Fricke (1934), in solutions in equilibrium with oxygen at pressures down to 4 cm. Hg. The influence of the concentration of dissolved oxygen on the yield of hydrogen peroxide resulting from the irradiation of water by X- and α-radiation has recently been much more fully investigated by Bonet-Maury &

* Cf., however, footnote to p. 55.

† It is noteworthy that Spicer (1935) observed that an aqueous solution of ferric chloride was completely reduced by a high, though unknown, dose of α-radiation, whereas when ferrous or ferric solutions are exposed to X-radiation an equilibrium seems to be reached at which only about 10% of the iron is in the ferrous form.

Lefort (1948). The irradiation of fully oxygenated water by low doses of α-radiation—i.e. doses such that the radiation does not appreciably decompose the hydrogen peroxide formed—would have been expected to yield appreciably more hydrogen peroxide than pure water, since the hydrogen atoms as well as hydroxyl radicals could contribute to the total hydrogen peroxide production. The experiments of Bonet-Maury & Lefort, however, do not confirm this expectation.

(2 *d*) *Carboxypeptidase dissolved in oxygenated water*

It is not yet known whether the inactivation of carboxypeptidase involves interaction with hydrogen atoms or hydroxyl radicals, or, indeed, with either. The low α-ray yield compared with the X-ray yield does, however, point to the less successful competition of enzyme in the case of the high ion density radiation for radicals of some kind which are capable of elimination by mutual interaction. Yet it can be seen from general considerations that the low α-ray yield which we have observed cannot be wholly accounted for by a consideration of recombination within the column surrounding the α-ray track. Since the X-ray ionic yield $\left(\frac{M}{N}\sim\frac{1}{5}\right)$ is independent of enzyme concentration above 0·003% weight of enzyme in solution (figure 4) we conclude that at these concentrations virtually the whole of the ions or radicals formed by X-rays are eliminated by collision with carboxypeptidase and only a negligible proportion by the alternative reaction. If the fact that the α-ray yield is only one-twentieth of the X-ray yield is taken to indicate that, on account of the high ion density within the α-ray column, only one-twentieth of the ions or radicals make collisions with enzyme molecules before they are eliminated, the probability of encounter between radicals and enzyme molecules must be correspondingly low. That probability should therefore be proportional to the concentration of enzyme molecules. It was with this point specially in mind that the influence of enzyme concentrations on α-particle ionic yield was studied. The results, as we have seen (p. 48) are incompatible with the hypothesis of proportionality between yield and concentration.

We know, however, that a proportion of the α-particle energy will be transformed into δ-rays, which travel clear of the main column of ionization. If the radius of the column is 0·015μ the required δ-ray energy is 0·4 kV. Those δ-rays which have an energy greater than 1 kV will be expected to have an ionic efficiency comparable with that of an X-ray recoil electron and should, therefore, make a contribution to the total inactivation of enzyme which is approximately independent of enzyme concentration. δ-rays of energy between 0·4 and 1 kV will be expected to have good ionic efficiency for a solute which reacts with hydrogen atoms but not for one which reacts with hydroxyl radicals. We do not know to which class carboxypeptidase belongs.

In order to obtain an upper limit for the δ-ray contribution, we have estimated* the ionization produced by all δ-rays, irrespective of energy, at distances greater than 0·015μ from their point of origin as a fraction of the total ionization produced by the α-particle. This fraction varies with the 'instantaneous' energy of the α-particle in the manner shown in figure 7, from which it is easy to compute that the mean value for radon and Ra A α-

* We have used Lea's computed δ-ray energy distributions and energy-range data throughout (Lea 1946, p. 30).

particles is 8·9% and that for Ra C′ α-particles 10·8%. For the α-rays from radon in solution the mean may therefore be taken as about 9·8%. The δ-rays outside the column of highly concentrated ionization along the α-ray track may therefore contribute as much as 10% of the total ionization. If this ionization is chemically as effective as that along the recoil electron tracks, the overall efficiency of α-radiation would never be expected to be less than 10% of that of X-rays. The calculation has also been carried out for the case in which only δ-ray ionization at more than 0·04 μ from the axis of the column, or three times the estimated column radius, has been considered. The δ-ray contribution, as a function of 'instantaneous' α-particle energy, is shown in figure 7, from which the mean value for the α-particles from radon in solution is found to be about 4%.

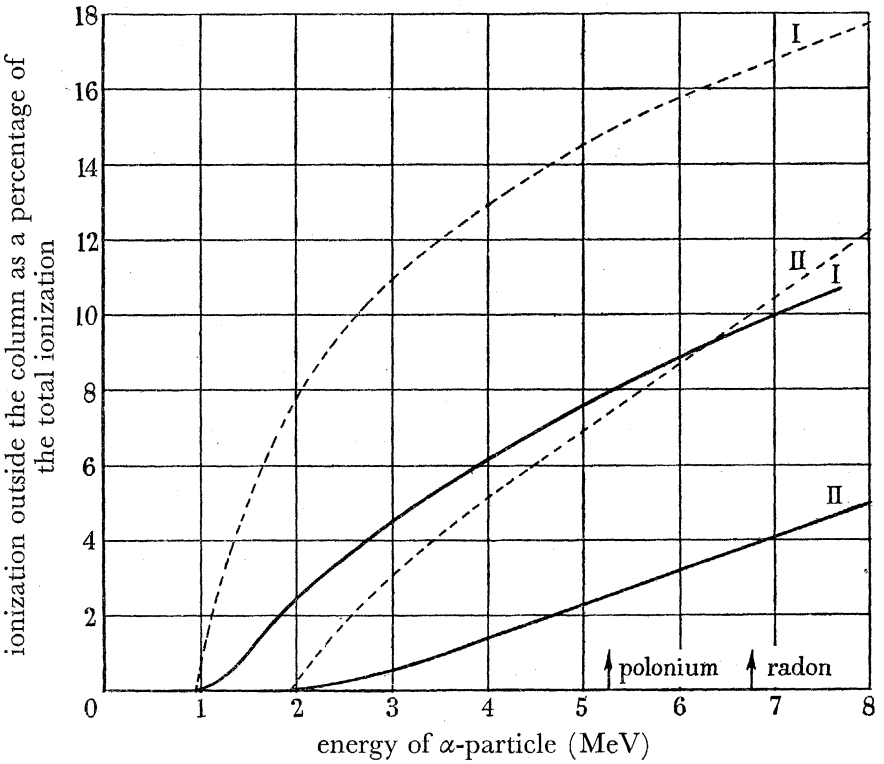


FIGURE 7. δ-ray ionization. The ordinates show the ionization produced by all δ-rays at distances greater than an arbitrary distance, d, from their point of origin, expressed as a fraction of the total α-particle ionization. Broken lines, abscissae refer to the instantaneous energy of the α-particle. Full lines, abscissae refer to the initial energy of the α-particle. In each case curve I refers to $d = 0{\cdot}015\,\mu$ in water and curve II to $d = 0{\cdot}04\,\mu$ in water.

Thus, if the radicals concerned with the inactivation of enzyme are derived from the negative ions, the δ-ray efficiency might approach that of X-radiation and show little dependence on concentration over the range studied, thus introducing a term of the order 4 to 10% into the overall α-ray inactivation efficiency which varies only slightly with enzyme concentration. If the radicals concerned are derived from the positive ions the δ-ray concentration would be expected to be somewhat smaller.

Referring to the experimental results (figure 6 *a* and *b*) it will be seen that δ-ray ionization alone might account for the observed α-ray inactivation, especially as inactivation by such low energy electrons may show some dependence on enzyme concentration. On the other

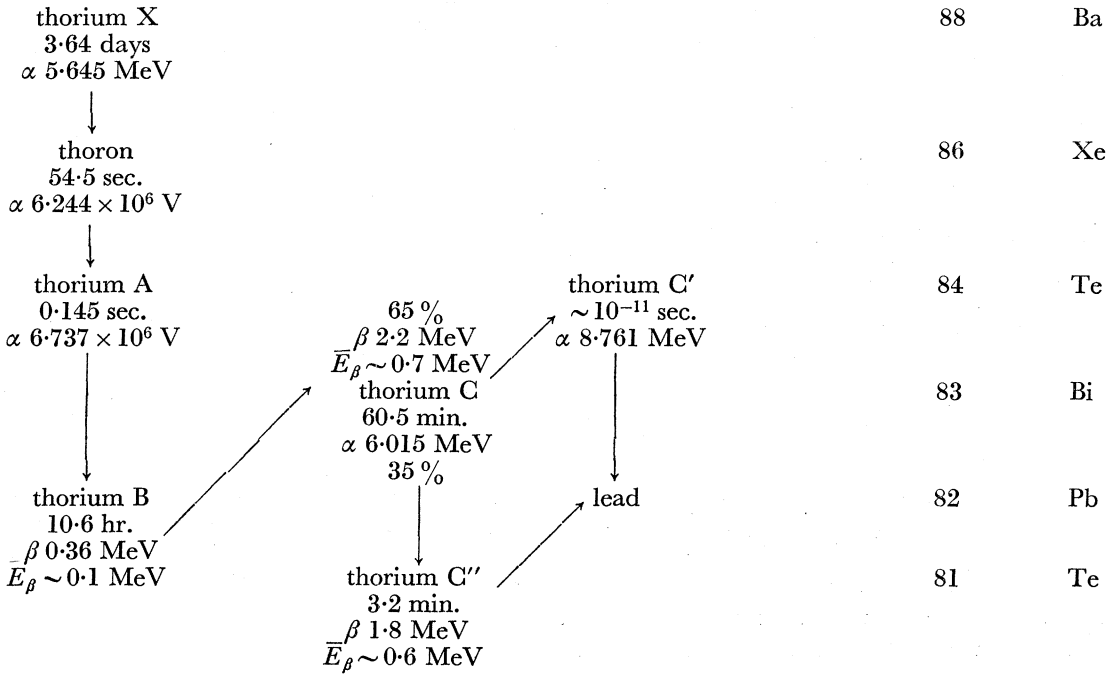
hand, the experimental results do not exclude the possibility that there is a term in the total inactivation which is proportional to concentration and might be ascribed to the main column of ionization. The form of the δ-ray ionization curves (figure 7) suggests that further experiments with low energy α-rays would be of considerable interest. The difference between polonium and radon α-particles would be difficult to establish with certainty. The slow neutron disintegration of lithium and boron provides such low energy α-rays but the influence of the δ-rays would in these cases probably be masked by the presence of other ionizing particles. Boron disintegrated by pure thermal neutrons would be expected to have an extremely small ionic efficiency at low concentrations of solute.

It is clear that, even under conditions in which radicals are predominantly eliminated by interaction with enzyme, the expansion of the column may be quite limited, and appreciable concentrations of radicals may only exist in the immediate vicinity of the track, so that only a small fraction of the enzyme in solution may be exposed to inactivation. Lea has shown* that if, as appears to be true in the case of carboxypeptidase, inactivated enzyme has approximately the same efficiency in eliminating radicals as the unchanged enzyme, then the α-ray ionic yield limited in this way by the self-protection of the enzyme will be independent of enzyme concentration.

We incline to the view that these conditions do not, in fact, prevail in our α-ray experiments. It appears most probable that the small observed α-ray ionic yield is due principally to δ-ray ionization, and that the majority of the labile product has no opportunity of reacting with enzyme molecules because it is rapidly eliminated within the α-ray column by alternative reactions.

Appendix

The inactivation of carboxypeptidase by the α-radiation from thorium X



* Private communication.

From the above disintegration scheme it will be evident that, on account of the relatively long period of thorium B (10·6 hr.), enzyme in solution with pure thorium X may be exposed for periods of the order of 2 hr. to α-radiation accompanied by very much smaller intensity of β-radiation than would be possible by the use of dissolved radon. In the experiments here described, the β-ray dose was only 0·03 % of the α-ray dose and the inactivation attributed to the β-radiation about 4 % of that attributed to the α-radiation. This proportion is much smaller than was attained even in the best radon experiments. Unfortunately, all thorium X solutions used were chemically toxic to the enzyme.*

A great deal of effort was spent to discover the source of the toxic effect. It is sufficient here to summarize the results of this unsuccessful search.

No toxic effect was produced by: (1) concentration of $CaCl_2$ below 1·6 molarity; (2) lead (which, as the final decay product of thorium X might act as a poison) used as $Pb_2OH(C_2H_3O_2)_3$ of $1{\cdot}24\times10^{-7}$ molarity; Ra D (radiolead) in similar concentrations; (3) thorium as $Th(NO_3)_4 \,.\, 4H_2O$ of 2×10^{-8} molarity; (4) decayed radon solution.

One has, therefore, to assume that there are toxic substances present derived from the original raw material from which radiothorium solutions are manufactured, which are not removed by the routine purifications.

The Th X had been prepared by chemical separation from a stock solution of radiothorium, with a known admixture of thorium which is isotopic with radiothorium. The solution supplied was then treated as follows.

In order to remove any decay products present in the thorium X solution 10 ml. of a thorium X solution were diluted to about 20 ml. and heated to near boiling, when 0·3 ml. of a 61 % w/v $FeCl_3$ solution and 3·5 ml. of concentrated ammonia were added. The resulting $Fe(OH)_3$ precipitate was filtered off, washed with ammoniacal water by resuspending and subsequent centrifuging. The supernatant clear filtrate was added to the filtrate from $Fe(OH)_2$ and evaporated to dryness, and all ammonia salts removed by gently heating until the residue was white. This residue was dissolved in 0·6 % HCl, again evaporated to dryness and finally gradually dissolved by hot water to a total volume of about 10 ml. (a small amount of a whitish residue was centrifuged off), and made up to 19 ml. Addition of 12 drops of N/10 NaOH was required in order to make the solution pink to phenolphthalein.

When finally mixed with enzyme, it is estimated that the concentration of the various substances present was:

enzyme	$1{\cdot}5\times10^{-7}$ molar
thorium X	$2{\cdot}8\times10^{-9}$ molar
$CaCl_2$	$0{\cdot}11_2$ molar
thorium	less than 10^{-9} molar†
radiothorium (isotopic with thorium)	less than 3×10^{-15} molar†

* We wish to express our great indebtedness to Dr Meyer of Brimsdown Chemical Works Ltd. for the very valuable advice he gave us in attempts to discover and eliminate the substance toxic to the enzyme.

† Three months after the irradiation had been carried out the activity of the enzyme solution, which had contained thorium X, was examined for γ-ray activity, which would have indicated the presence of radiothorium, since the activity associated with the thorium X would by this time be beyond the limits of detection.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Irradiation with X-rays caused the same percentage inactivation of enzyme, already partially inactivated chemically by thorium X, as of pure enzyme. It appeared, therefore, that the chemical and the irradiation inactivations proceed independently and consequently that some weight should be attached to this experiment. The results will be evident from table 5.

TABLE 5

solution	treatment	X-ray dose in roentgens	α-ray dose in energy units	β-ray dose in energy units or roentgens	observed enzyme activity [C.P.u.]$^{\text{P.D.E.}}$ per ml. ($\times 10^{-6}$)
A	untreated enzyme	—	—	—	30·2
B	X-rays only	700	—	—	8·4
C	thorium X for 151 min.	—	12,600	34	1·36
D	thorium X only for 4 min.	—	334	0·9	8·37
E	thorium X for 9 min. + X-rays	700	750	2	1·90

The γ-ray activity of the several solutions was followed from $t = 1$ to $t = 41$ hr. from the commencement of the irradiation. The growth in activity agreed very well with expectation and enabled the concentration of the various radioactive bodies present at any time to be calculated, and hence the α- and β-ray doses delivered in each of the solutions C, D and E.

Comparing solutions A and B we find, for irradiation of pure enzyme by X-rays,

$$\frac{A}{A_0} = e^{-2\cdot05\times10^{-3}\,D}.$$

Similarly, comparing solutions D and E and allowing for the small α- and β-ray contributions

$$\frac{A}{A_0} = e^{-1\cdot83\times10^{-3}\,D}.$$

The ionic yield of X-radiation in the presence of thorium X, represented by the exponent $1\cdot83\times10^{-3}$ is therefore not greatly different from that represented by $2\cdot05\times10^{-3}$ for pure enzyme, even though the enzyme is reduced in activity (chemically) to 29 % of its normal value.

Comparing solutions C and D and allowing for the small β-ray contribution, the inactivation due to α-radiation may be computed to be

$$\frac{A}{A_0} = e^{-1\cdot42\times10^{-4}\,D},$$

so that the relative efficiency of α- and X-radiation is given by

$$\eta_x^{\alpha} = \frac{1\cdot417\times10^{-4}}{2\cdot05\times10^{-3}} = 0\cdot069.$$

The result of this experiment is therefore to indicate an ionic efficiency of α-radiation about 7 % of that of X-radiation when the enzyme concentration is $c = 0\cdot61\times10^{-5}$. This agrees well with the results of the radon experiments.

A high pressure ionization chamber was used for this purpose and the standard deviation of the measurement was $\pm 6\times10^{-4}\,\mu$C. No activity was observed. If the amount of radiothorium present is therefore taken as not exceeding $6\times10^{-4}\,\mu$C, we can estimate from the known ratio of thorium to radiothorium in the original stock solution that the concentration of thorium in the enzyme solution could not have exceeded 10^{-9} molar.

One of us (L. H. G.) gratefully acknowledges the financial assistance given by the British Empire Cancer Campaign to the Research Department of the Mount Vernon Hospital, Northwood.

References

Allsopp, C. B. 1944 *Trans. Faraday Soc.* **40**, 79.
Alper, T. 1932 *Z. Phys.* **76**, 172.
Anson, M. L. 1937*a* *J. Gen. Physiol.* **20**, 663.
Anson, M. L. 1937*b* *J. Gen. Physiol.* **20**, 777.
Bonet-Maury, P. & Lefort, M. 1948 *C.R. Acad. Sci., Paris*, **226**, 1363, 1445.
Cameron, A. T. & Ramsay, Wm. 1907*a* *J. Chem. Soc.* **91**, 931.
Cameron, A. T. & Ramsay, Wm. 1907*b* *J. Chem. Soc.* **91**, 1266, 1593.
Cameron, A. T. & Ramsay, Wm. 1908 *J. Chem. Soc.* **92**, 966, 992.
Dainton, F. S. 1948 *J. Phys. Colloid Chem.* **52**, 490.
Dale, W. M. 1940 *Biochem. J.* **34**, 1367.
Dale, W. M. 1942 *Biochem. J.* **36**, 80.
Dale, W. M. 1943 *Brit. J. Radiol.* **16**, 171.
Dale, W. M., Meredith, W. J. & Tweedie, M. C. K. 1943 *Nature*, **151**, 280.
Duane, W. & Scheuer, O. 1913 *Le Radium*, **10**, 42.
Fricke, H. 1934 *J. Chem. Phys.* **2**, 556.
Frilley, M. 1947 *Brit. J. Radiol.*, Supplement No. 1, p. 46.
Gray, L. H. 1944 *Proc. Camb. Phil. Soc.* **40**, 72.
Gray, L. H. 1946 *Brit. Med. Bull.* **4**, 11.
Gray, L. H., Mottram, J. C., Read, J. & Spear, F. G. 1940 *Brit. J. Radiol.* **13**, 371
Gray, L. H. & Read, J. 1942 *Brit. J. Radiol.* **15**, 320.
Jaffé, G. 1913 *Ann. Phys., Lpz.*, **42**, 303.
Kara-Michailova, E. & Lea, D. E. 1940 *Proc. Camb. Phil. Soc.* **36**, 101.
Klemperer, O. 1927 *Z. Phys.* **45**, 225.
Lanning, F. C. & Lind, S. C. 1938 *J. Phys. Chem.* **42**, 1229.
Lea, D. E. 1946 *Actions of radiations on living cells.* Cambridge Univ. Press.
Lea, D. E. 1947 *Brit. J. Radiol.*, Supplement No. 1, p. 59.
Meredith, W. J. & Stephenson, S. K. 1943 *Brit. J. Radiol.* **16**, 239.
Nurnberger, C. E. 1936 *J. Chem. Phys.* **4**, 697.
Nurnberger, C. E. 1937 *Proc. Nat. Acad. Sci., Wash.*, **23**, 189.
Rutherford, E., Chadwick, J. & Ellis, C. D. 1930 *Radiations from radioactive substances.* Cambridge Univ. Press.
Spicer, G. W. 1935 *Trans. Faraday Soc.* **31**, 1706.
Stenstrom, W. & Lohmann, A. 1928 *J. Biol. Chem.* **79**, 673.
Svedberg, T. & Brohult, S. 1939 *Nature*, **143**, 938.
Weiss, J. 1944 *Nature*, **153**, 748.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES