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γ-RADIOLYSIS OF AQUEOUS CYSTEINE

by

VERNA GAYE WILKENING

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

γ-RADIOLYSIS OF AQUEOUS CYSTEINE

submitted by Verna Gaye Wilkening in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Date Oct. 27, 1966.

ABSTRACT

Previous work had established the importance of thiol and disulfide groups in protection of living organisms against radiation damage.[51,55] As an aid in elucidating the mechanism of *in vitro* γ-radiolysis, the radiation chemistry of aqueous solutions of cysteine and two simpler thiols has been investigated. The data obtained have been correlated with and interpreted in terms of the modern concepts of the radiation chemistry of water. One method used in this investigation was that of competition kinetics.

It was found that for initial cysteine concentrations of 10^{-3} M and lower the G-values of all products were dose dependent. Since mechanistic conclusions can usually be drawn only from dose independent yields or true initial yields, discussion of the data emphasized the more concentrated solutions where the range for dose independence was greater. In all cases, the radiolytic yields have been reported only for initial slope data, and given as G-values. The major products identified and measured quantitatively were cystine, hydrogen, hydrogen sulfide, and alanine. Cystine and hydrogen sulfide were determined spectrophotometrically, hydrogen from pressure difference following gas diffusion through a heated palladium thimble, and alanine by means of an amino acid analyzer.

The G-values of all the products were pH dependent. This dependence was discussed in terms of reactions with the reducing species, H and e^-_{aq}, and the oxidizing species, OH, formed in irradiated water.

A mechanism has been suggested to account for the products formed in the radiolysis of dilute, air-free thiol solutions. For 10^{-2} M solutions, the G-values for hydrogen and hydrogen sulfide were in reasonable agreement with values predicted by the mechanism over the pH range 0 - 6.

The data obtained for 10^{-2} M solutions appeared to indicate that, in neutral solutions, hydrogen sulfide was formed almost entirely from solvated electron attack on the sulfhydryl group of cysteine:

$$e^-_{aq} + RSH \longrightarrow SH^- + R \qquad (54)$$

and that very little came from attack of "residual" hydrogen atoms:

$$H + RSH \longrightarrow H_2S + R \qquad (43)$$

It was concluded from competition kinetic studies that reaction (54) was rapid and almost diffusion-controlled for cysteine, and also for the related thiols, methyl mercaptan and 2-mercaptoethanol. The implications of this in radiation biology have been briefly examined.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

1.1 Radiation Chemistry

Radiation chemistry may be defined as the study of the chemical effects produced in a system by the absorption of ionizing radiation. Studies of the chemical effects of X-rays began immediately after their discovery by Roentgen in 1895. The subsequent work on radioactive nuclides by Becquerel and the Curies added impetus to this research. Work on the large scale release of nuclear energy during and following World War II stimulated interest in the effect of radiation on matter, particularly living matter. At this time the name "radiation chemistry" was applied to the subject to distinguish it from the study of the chemistry of the radioactive nuclides, which retained the name of "radio-chemistry".

With the development of atomic energy programs, a variety of particle accelerators became available. These have been used to provide high energy radiation for various specific studies in radiation chemistry. Also of great importance has been the increasing availability of artificial radioactive isotopes, such as Co^{60} and Sr^{90}, which provide intense, relatively cheap sources of radiation.

1.1.1 Interaction of radiation with matter

Some knowledge of the processes by which radiation interacts with matter is essential to the understanding of the phenomena of radiation chemistry, since the chemical effects are a direct consequence of the absorption of energy.[1] Ionizing radiation consists

of high energy photons or particles which possess energy in excess of the ionization potentials of atoms and molecules. It may be divided into three major groups according to source: radiation from radioactive nuclei (α-, β-, and γ-rays), charged particle radiations (electrons, protons, deuterons, etc.), and electromagnetic radiation of short wavelength (X-rays with wavelength less than 250 Å, or energy greater than about 50 eV per quantum). These radiations are characterized by the fact that they lose energy on penetrating matter, primarily by causing ionization and electronic excitation. Each photon or particle can ionize or excite a large number of the molecules along its track. These high-energy photons or particles are not selective and may react with any molecule in their path, raising it to any one of its possible ionized or excited states. As a result, the various ionized or excited species react to give a wide variety of products. This is in marked contrast to the relatively small number of distinguishable products obtained from most photochemical reactions. Radiation-induced reactions may be further complicated by mutual reactions of the ionized and excited molecules, since they are initially produced in high local concentration, especially in condensed phases.

It is outside the scope of this thesis to present a detailed discussion of the radiation physics of all types of radiations. However, the primary energy loss processes of electromagnetic radiation and fast electrons are pertinent to this investigation. Therefore they will be discussed briefly.

In contrast to charged particles, photons tend to lose

relatively large amounts of energy when they interact with matter. The reduction in electromagnetic radiation intensity, dI, on passing through a small thickness, dx, of absorber is given by

$$dI = - I_i \mu dx$$

In the above expression, I_i is the intensity of incident radiation, and μ is the total linear absorption coefficient. Not all incident photons interact with a finite thickness of absorber. The transmitted radiation travels in the original direction with no energy change.

The linear absorption coefficient is thus the fraction of incident photons diverted from the beam by unit thickness of absorber. It is a constant for a given material and for radiation of a given energy. It is the sum of a number of partial coefficients, representing the various processes of absorption. These processes are the photoelectric effect, the Compton effect, pair production, coherent scattering, and photonuclear reactions. The first three are the most important, although the relative importance of each process depends on the photon energy and the atomic number of the absorbing material.

(i) photoelectric effect:

The interacting γ-ray (or X-ray) is completely absorbed and an electron is ejected from an atom or molecule. There is conservation of both momentum and energy. The ejected electron departs with an energy equal to the difference between the γ-ray energy and the binding energy, B.E., of the electron in the atom

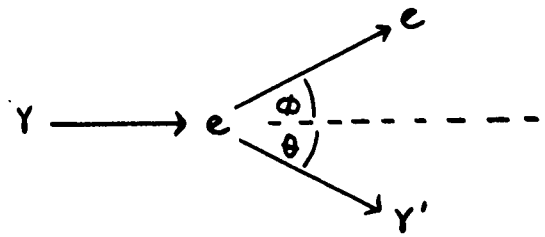
or molecule:

$$E_e = E_\gamma - B.E._e$$

In low atomic weight materials photoelectric absorption is relatively unimportant for energies above 1 MeV.

(ii) Compton effect:

The incoming γ-ray interacts with either a free or loosely bound (regarded as free) electron:



The energy and momentum of the incident photon are shared between the scattered photon and the recoil electron.

$$E_\gamma = E_e + E_{\gamma'}$$

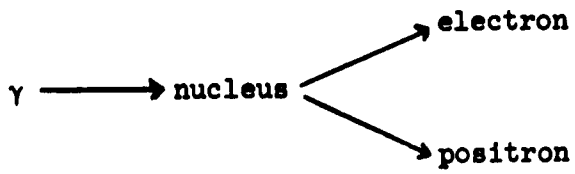
The most probable electron energies are those near zero and near the maximum energy ($\theta = 180°$). The latter situation is favoured when the energy of the incident photon is high. The expression which gives the probability of the photon being scattered with a definite energy or direction was derived by Klein and Nishina.[1]

The total Compton absorption coefficient refers to the probability of Compton interaction as a whole. It is the sum of the Compton scattering coefficient and the true Compton absorption coefficient. The former refers to energy transferred

to the deflected photons, the latter to energy transferred to the recoil electrons. The ratio of true to scatter absorption coefficients varies with the energy of the incident photons. The Compton effect is important for photon energies from 1 - 5 MeV in high atomic number materials, and over a much wider range in low atomic number materials. For example, it is the only important process occurring when Co^{60} γ-rays interact with water.[2]

(iii) pair production:

In this process a positron-electron pair is produced within the medium:



For this process to occur, the energy of the photon must be at least equivalent to the sum of the rest masses of the two particles, i.e. $\geq$ 1.02 MeV.

$$E_\gamma = E_e + E_p + 2 m_o c^2$$

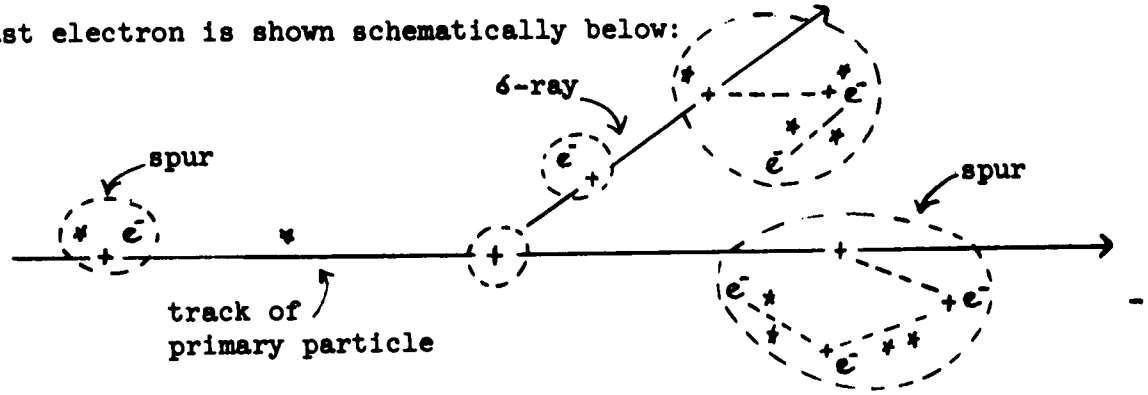
The electron and positron are energetic, and in losing their energy, cause ionization and excitation of the medium. The positron is eventually destroyed by combining with an electron, producing in the process two photons of 0.51 MeV each. These are called annihilation radiation, and will lose their energy via the Compton or photoelectric effect.

Electromagnetic radiation, absorbed by any of the above

three processes, gives rise to fast electrons within the medium. It may be assumed that the chemical effects of high-energy radiation are due almost entirely to the fast electrons generated in the system. They may interact with either the nucleus or the electrons of an atom. Interaction with the nucleus produces X-rays (bremmstrahlung), which in turn produce more fast electrons. Interaction with an electron of an inner shell results in ejection of the orbital electron. The vacancy in the positively charged ion is filled by an outer electron dropping down into the lower level. This process is accompanied by the emission of an X-ray photon or another electron (Auger effect). Interaction with an electron of an outer shell results in either ionization or excitation. The overall result of the absorption of ionizing radiation by matter is the formation of tracks of excited and ionized species. Fast charged particles of different types and energies will lose energy at different rates, and consequently will form tracks that may be densely or sparsely populated. The linear rate of energy transfer along the track of the particle is known as the LET, and is useful in evaluating the overall chemical effect. Such track effects are particularly important in liquids, where the active species are hindered from moving apart by the proximity of other molecules.

When ejected electrons possess energies which are considerable fractions of the energy of the original fast electron, they are called secondary electrons. Their tracks will branch off from the primary track and be similar to those of other electrons of the same energy. If their energy is lower than that of secondary electrons

but greater than 100 eV, they are referred to as delta rays. If their energy is lower than 100 eV, but greater than the ionization potential of the medium, their range in liquid or solid materials will be relatively short. Any ionizations which they produce will be situated close to the original ionization and will result in a small "cluster" or spur. The average spur contains 2 - 3 ion pairs and some excited molecules. It corresponds to an energy loss of about 100 eV. The distribution of ions and excited molecules in the track of a fast electron is shown schematically below:



Once the electrons have slowed down to thermal energies they will either neutralize a positive ion directly or be captured by a neutral molecule to form a stable negative ion. This will eventually neutralize a positive ion.

1.1.2 G-values

The yields of radiation-induced reactions were originally expressed in terms of the ionic yield, M/N, which is the number of molecules formed or destroyed per ion-pair produced. However, since ionization can be measured accurately only in the gas phase, it is now common practice to express yields in terms of the number of molecules changed (formed or destroyed) per 100 eV of energy absorbed in

the system. The symbol G is used to denote these yields. Thus G(X) refers to the number of molecules of product X formed on irradiation per 100 eV of energy absorbed, and G(-Y) refers in the same way to the loss of material Y that is destroyed on irradiation.

1.1.3 Dosimetry

In order to evaluate yields and hence the efficiency of radiation in bringing about chemical reactions, it is necessary to find the rate of energy absorption in the medium. The measurement of energy absorption is called radiation dosimetry.

The original unit of absorbed dose was the roentgen, defined as "the quantity of X- or γ-radiation such that the associated corpuscular emission per 0.001293 g of air produces, in air, ions carrying 1 esu of quantity of electricity of either sign."[3] One cubic centimeter of any other medium placed in the same position relative to the same source would absorb energy in the ratio of its volume absorption coefficient relative to that of air. Since the roentgen is applicable only to X- or γ-radiation, another unit called the rad was defined. This is now the official unit of absorbed dose,[1] and is defined as 100 ergs/g. It is also common practice in radiation chemistry to give the absorbed dose in units of electron volts per gram or per cubic centimeter.[1] One rad is equivalent to 6.24×10^{13} eV/g.

With ionizing radiation, energy is dissipated in the medium by photoelectrons and Compton recoil electrons. In the latter there is a good deal of scattered radiation, only a portion of which

may be absorbed. However, it is possible to calculate the ratio of the absorption coefficients of any two media for a given wavelength, provided their chemical composition is known.

In practice, most determinations of dose rate are based on measurements of the amount of ionization produced either in the reacting system itself, if gaseous, or in an air-filled ionization chamber. It is known that the mean energy dissipated in air at standard temperature and pressure by an electron in creating an ion-pair is 34.0 eV.[2] Hence the rate of ionization gives a measure of the energy absorbed.

Some of the difficulties of dosimetry may be avoided by using a radiation-induced chemical reaction as an integral dosimeter. To be suitable for this purpose the reaction should have a constant G-value over a wide range of intensities and types of radiation; the extent of reaction should be proportional to dose over a wide range, and be easily measurable; and the reagents employed should be convenient to prepare and store. The chemical dosimeter that best fulfills these conditions uses the oxidation of Fe(II) to Fe(III) in air-saturated, dilute sulfuric acid solution. Fricke[1] proposed this system in 1929 as an X-ray dosimeter, and now it is commonly referred to as the "Fricke dosimeter". The G-value for this reaction has been carefully determined by several techniques to be 15.6.[1] Weiss *et al*[4] have given the procedure for preparing and using this dosimeter. In earlier work it was customary to determine the ferric ion yield spectrophotometrically by its absorption at 304 mμ.

The extinction coefficient at this wavelength is 2174 at 24° C, and it increases by 0.7% per degree between 20° and 30° C. Recently, it has been reported that the sensitivity of the method can be improved by measuring the absorption at 224 mμ, where the extinction coefficient is 4565.[5] The temperature dependence at this wavelength is only 0.1% per degree.

A mechanism for the radiation-induced oxidation of ferrous sulfate in the Fricke dosimeter described above has been deduced. For reasons to be explained in the next section, the radiation-induced decomposition of water may be represented by the equation:

$$H_2O \xrightarrow{\sim\sim} H + OH + H_2 + H_2O_2 \qquad (1)$$

The symbol $\xrightarrow{\sim\sim}$ is to be read "under the influence of high-energy radiation gives". The hydrogen and hydrogen peroxide are referred to as molecular products, while H and OH are designated radical products. The yields of these various species are referred to as $G^M_{H_2}$, $G^M_{H_2O_2}$, G_H, and G_{OH}, respectively. The subsequent steps are believed to be as follows:

$$OH + Fe^{++} \longrightarrow Fe^{+++} + OH^- \qquad (2)$$

$$H + O_2 \longrightarrow HO_2 \qquad (3)$$

$$H^+ + HO_2 + Fe^{++} \longrightarrow Fe^{+++} + H_2O_2 \qquad (4)$$

$$H_2O_2 + Fe^{++} \longrightarrow Fe(OH)^{++} + OH \qquad (5)$$

The OH radical produced in step (5) reacts according to (2). Thus, for every water molecule decomposed, four Fe^{++} ions are oxidized, and this agrees with experimental observations.

The topic of radiation dosimetry will not be discussed further here. Greater detail may be found in references (6) and (7).

1.2 Radiolysis of Water

An explanation for the action of radiation on pure water is fundamental to an understanding of the effects found in aqueous solutions. Both oxidation and reduction of dissolved substances in irradiated aqueous solutions were observed as early as 1914.[1] It was suggested then that free radicals formed from the water must be responsible for the chemical action of the radiation. Theoretical and experimental work on the subject up to 1965 has been summarized by several authors.[1,8,9,10,11,12,13,14].

As pointed out in the previous section, ionization and excitation are the first events which occur upon the absorption of ionizing radiation by matter. What happens after ionization is still subject to conjecture. Subsequent reactions will depend to a large extent on the distance the secondary electron has travelled from the parent ion before it is reduced to thermal energy. Two important models, the Samuel-Magee and the Lea-Gray, have been put forward.

Samuel and Magee[15] calculated that before thermalization can occur ($\sim 10^{-13}$ sec.), a 10 eV electron could travel a distance of approximately 20 Å from the parent ion. At this distance the electron would still be within the electrostatic field of the parent ion.

The electron would therefore return to the parent ion and react with it. The product of the neutralization reaction would be a highly excited water molecule:

$$H_2O^+ + e^- \longrightarrow H_2O^{**} \longrightarrow H + OH \qquad (6)$$

which would decompose into a hydrogen atom and a hydroxyl radical. These radicals would possess enough energy to escape the solvent cage.

An alternative model was proposed by Lea[1] and Gray[1] who estimated that the secondary electron would travel about 150 Å before thermalization. Thus the ion and the electron would react independently with the solvent:

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH \qquad (7)$$

$$e^- + H_2O \longrightarrow H + OH^-_{aq} \qquad (8)$$

The hydroxyl radical, from reaction (7), would be formed near the site of the original ionization; the hydrogen atom would be produced in reaction (8) at some distance from the parent ion. It should be noted that reaction (8) is endothermic and can occur only in liquid water where the solvation energy of OH^- is available.

Platzman[1] developed a model which is similar to that of Lea and Gray, except that the distance between the secondary electron and the parent ion was assumed to be only 50 Å. At this distance the electrostatic attraction would be very small. Platzman suggested that since the lifetime of the electron with respect to reaction (8) is of the order of 10^{-11} sec., the electron might not undergo this reaction

but might become solvated instead.

In recent years it has been shown by spectroscopic and other techniques that "solvated electrons" are the main reducing species formed in water by γ-ray or fast electron radiolysis. The major oxidizing species is considered to be the hydroxyl radical. In addition there is evidence for a third species, the identity of which is not established. This species reacts similarly to a hydrogen atom in some solutions[16] and to an electron in others.[17] Some authors[18] have suggested that this unknown species is some form of excited water molecule. The experimental evidence available for the formation of solvated electrons, hydrogen atoms, and excited water molecules in irradiated water will be briefly presented and discussed.

The fate of the radical and molecular products depends on the concentration and reactivity of the solutes present. In a closed system and in the absence of any other solute, the radical and molecular products tend to react together, setting up a chain reaction

$$H + H_2O_2 \longrightarrow H_2O + OH \qquad (10)$$

$$OH + H_2 \longrightarrow H_2O + H \qquad (11)$$

In an open system, molecular hydrogen can escape, and the chain reactions do not occur. In the presence of a highly reactive solute at a concentration greater than about 10^{-6} M, the radicals diffusing out of the spurs will not react with one another or with the molecular products but with the solute. Therefore, radical and molecular yields

can be determined experimentally by irradiating suitable aqueous solutions.

Intensive study of the hydrogen - oxygen - hydrogen peroxide aqueous system has given G-values for the various species formed in the radiolysis of neutral water[11] as:

$$G_{red} = 2.75,\ G_{OH} = 2.2,\ G^{M}_{H_2} = 0.45,\ G^{M}_{H_2O_2} = 0.7$$

G_{red} refers to the number of reducing species (e^-_{aq} or H) formed per 100 eV.

Barr and Allen[19] were among the first to discover chemical evidence for the existence of solvated electrons. They suggested that the hydrogen atom produced in the free radical oxidation of hydrogen was different from the reducing species produced in the radiolysis of water. The hydrogen atom formed from hydroxyl radical attack on hydrogen reacts much more rapidly with oxygen than with hydrogen peroxide, whereas that formed directly from irradiated water reacts at comparable rates with the two substances. The suggestion was made that one form was atomic hydrogen and the other a basic or an acidic form of the hydrogen atom - i.e. either a solvated electron or the H_2^+ ion.

Hayon and Allen[20] studied the γ-radiolysis of aqueous solutions of monochloroacetic acid at various concentrations and values of pH. The products were chloride ion and hydrogen gas. The kinetic evidence indicated that H^+ and the solute were competing for the reducing species formed in irradiated water:

$$H^+ + \text{reducing species} \longrightarrow H \qquad (12)$$

$$\begin{matrix} CH_2COOH \\ | \\ Cl \end{matrix} + \text{reducing species} \longrightarrow Cl^- + \cdot CH_2COOH \qquad (13)$$

To explain these results the authors concluded that H and the reducing species were different. There are other examples of experiments which appeared to require two different kinds of reducing species.[1,21]

Until 1961, it was not possible to determine from the existing data whether the two reducing forms were e^-_{aq} and H, or H and H_2^+, or possibly some other unidentified entity. The most convincing evidence that the reducing species in neutral solutions was the solvated electron came from relative rate determinations made in the presence of chemically inert salts.[22,23] At low salt concentrations, rate constants for reactions between ions of similar charge increase with increasing ionic strength, while rate constants for reactions between ions of opposite charge decrease with increasing ionic strength.[24] This kinetic salt effect was used by Czapski and Schwarz[22] to determine the charge on the reducing species formed in irradiated water. Their results indicated that this species had a negative charge, and from the slope of the ionic strength curves, it was shown that the charge was -1. That the reducing species possessed a negative charge was confirmed by Dainton and co-workers,[23] who studied the competition between silver ion and acrylamide. They concluded that at pH 4 the reducing species has a unit negative charge, while at pH 2 it is uncharged.

Finally, Hart and Boag[25] observed the absorption spectrum

of solvated electrons. Water was irradiated with a high intensity of fast electrons, and a transient coloration was observed. The spectrum of the irradiated water was found to be similar to that of the solvated electron found in liquid ammonia. Using a linear accelerator with pulses of 0.4 - 5.5 microseconds, and a flash lamp synchronized with the accelerator pulses, the workers recorded the spectrum photographically. An absorption peak due to the solvated electron was observed at approximately 700 mμ. To study the intensity and decay of the species the trace was followed on an oscilloscope.

Since the initial experiment of Hart and Boag, the pulsed radiolysis technique has been used a great deal to study reactions of solvated electrons in water and in other liquids.[26,27,28] By this method Dorfman[28] was able to conclude that in acid or neutral solutions, the following reaction occurred:

$$e^-_{aq} + H^+ \longrightarrow H \qquad (14)$$

In alkaline solutions, reaction (15)

$$e^-_{aq} + H_2O \longrightarrow H + OH^- \qquad (15)$$

occurred if the concentration of the solvated electron was low, and reaction (16) if it was high:

$$e^-_{aq} + e^-_{aq} \longrightarrow H_2 + 2\,OH^- \qquad (16)$$

The yield of the solvated electron was determined by Czapski and Allen[29] from a competition involving oxygen and hydrogen peroxide.

The result was $G_{e^-_{aq}} = 2.85 \pm 0.15$. This value was later confirmed from a study of the radiolysis of aqueous methanol - sodium nitrate solutions.[30] From studies in which oxygen and hydrogen were added as solutes to irradiated water,[31,32] a material balance showed $G_{red} = 2.8$. Hence, on the basis of the material balance it was concluded that all reducing radicals must be present as solvated electrons.

However, there was strong evidence that some hydrogen atoms were formed directly from water in neutral radiolysis. Allan and Scholes[33] and, later, others,[11] found that the addition of electron scavengers could reduce the observed hydrogen yield, $G(H_2)$, to 1.1 but no further. If $G^M_{H_2}$ is taken as 0.45, then the difference of 0.65 must be attributed to an independent yield of hydrogen atoms. In later work,[34,35] hydrogen atom scavengers were added and found to reduce $G(H_2)$ to 0.45.

Nehari and Rabani[36] studied the effect of pH on the yield of hydrogen in the X-radiolysis of solutions of various organic compounds in the presence of solvated electron scavengers. It was found that they too obtained a residual hydrogen atom yield of 0.48 which was pH independent from pH 2 - 13. In alkaline solutions they obtained an increase in the yield of a species which reacted with electron scavengers. Therefore, they postulated the reaction

$$H + OH^- \longrightarrow e^-_{aq} \qquad (17)$$

Kuppermann[37] recently calculated the theoretical value for residual hydrogen formation on the basis of a diffusion-kinetics model.

He concluded that the yield of hydrogen atoms which would arise from the spurs should be of the same order of magnitude as the experimental value.

On the basis of the foregoing information there seems to be little doubt that an independent yield of hydrogen atoms exists. However, if it is accepted, then the material balance for the primary yields for neutral irradiated water is no longer correct. The total number of reducing equivalents is given by

$$G_{red} + G_{"H"} + 2\,G^{M}_{H_2}$$

$G_{"H"}$ refers to the number of questionable hydrogen atoms formed per 100 eV, and the other symbols have their usual meanings. The above sum totals 4.3. The total number of oxidizing equivalents, which corresponds to

$$G_{OH} + 2\,G^{M}_{H_2O_2}$$

is only 3.6. Since the material balance obtained in acid solutions is excellent, the discrepancy observed in neutral solutions appears to be real. To explain this discrepancy it was suggested[11] that possibly there was another, as yet unidentified, oxidizing species. Other workers[38,39,40] had obtained data which could suggest the presence of higher radical yields than are usually quoted.

Recently, the yields of the hydroxyl radical, the solvated electron, and molecular hydrogen peroxide were re-determined[41] for neutral irradiated water. On the basis of the new values, the agree-

ment between the total number of oxidizing and reducing equivalents is excellent. Therefore there is in fact no need to postulate the existence of a new oxidizing species, as had been previously suggested. Dainton and co-workers[42] have also shown that the yields of oxidizing and reducing equivalents are equal. These data completely refute the earlier arguments against the existence of a "residual" hydrogen atom yield, which were based on a material balance calculated from earlier values of radical and molecular yields.

There has been, and there is still, much speculation as to the source of molecular hydrogen and the "residual" hydrogen atoms. Two schools of thought on the mechanism of formation of the hydrogen atoms are emerging. One favors a yield arising from inside the spurs or tracks, the other favors a yield arising outside the spurs.

In the spurs the initial concentration of radicals will be high and radical-radical reactions would be expected. In the absence of oxygen and below pH 10, the predominant radicals in irradiated liquid water are solvated electrons, hydrogen atoms, and hydroxyl radicals.[13] There are six possible combinations of these species:

$$e^-_{aq} + e^-_{aq} \longrightarrow ? \longrightarrow H_2 \qquad (18)$$

$$e^-_{aq} + H \longrightarrow ? \longrightarrow H_2 \qquad (19)$$

$$e^-_{aq} + OH \longrightarrow OH^- \qquad (20)$$

$$H + H \longrightarrow H_2 \qquad (21)$$

$$H + OH \longrightarrow H_2O \quad (22)$$

$$OH + OH \longrightarrow H_2O_2 \quad (23)$$

It has been demonstrated[43] that reactions (18) and (19) both produce hydrogen, although the mechanisms involved are not understood.

As the spurs or tracks expand, the probability of radical-radical reactions decreases and some of the original radicals diffuse into the bulk of the liquid. Allen[11] suggested that the independent yield of hydrogen atoms found in the radiolysis of neutral water must arise during the diffusion of the initial radiolysis products out of the spurs. Since the solvated electron is negatively charged and the hydroxyl radical is neutral, an equal number of hydronium ions must be formed in the spurs if electroneutrality is conserved. If it is argued that the molecular hydrogen yield, $G^M_{H_2}$, arises from recombination of solvated electrons in the spurs, as shown in reaction (18), and that $G^M_{H_2O_2}$ arises from recombination of hydroxyl radicals in the spurs, then the reaction of the solvated electrons with the hydronium ions should also occur:

$$e^-_{aq} + H_3O^+ \longrightarrow H + H_2O \quad (24)$$

As the three main radicals diffuse away from the site of formation, a yield of hydrogen atoms comparable to those of molecular hydrogen and hydrogen peroxide would therefore be expected. This is what is found experimentally, ($G_H = 0.55$). The magnitude of G_H which could be expected from a reaction within the spurs[37] and the size of the rate

constant[26] for reaction (24) supports the suggestion that "H" arises from a reaction inside the spurs.

There are several arguments against a residual hydrogen atom yield arising from the spurs. Dainton and Petersen,[18] studying the radiolysis of aqueous solutions of nitrous oxide, concluded that there are three forms of reducing species, which they identified as e^-_{aq}, H atoms, and H_2^+. They postulated the existence of an excited water molecule formed during the radiolysis of water, which, under the influence of acid, but not necessarily in reaction <u>with</u> the acid, could lead to H_2^+:

$$H_2O^* + H_3O^+ \longrightarrow H_2^+ + OH + H_2O \qquad (25)$$

The postulated existence of H_2O^* could explain the increase in G_{OH} and the "conventional" G_H which is found in the radiolysis of water at pH < 3.5.[12,44]

Excited water molecules have been postulated by other workers to account for the "residual" hydrogen atom yield. Thus, Hayon[45] studied the radiolysis of air-free aqueous solutions of hydrogen peroxide, and from his data suggested that the precursors of the hydrogen atoms are excited water molecules, the life-times of which were calculated to be at least 10^{-6} - 10^{-7} seconds. He proposed that the effect of oxygen could be to quench the excited water molecules, which would otherwise lead to formation of hydrogen atoms:

$$H_2O^* \longrightarrow H + OH \qquad (26)$$

This would imply that the "residual" hydrogen atom yield arises from outside the spurs.

Anbar[46] studied the effect of transition metal ions on the yield of hydrogen atoms in the radiolysis of 2-d-2-propanol in dilute, neutral aqueous solution. He found that the ions did not react with hydrogen atoms formed in acid solution, H, but did react with the so-called hydrogen atoms formed in neutral solution. Therefore, he concluded that H and the questionable hydrogen atoms are chemically different. There are two possibilities, e^-_{aq} or H_2O^*, which could be the precursors of the species formed in neutral solutions, the so-called "residual" hydrogen atoms. Anbar rejects the solvated electron on the grounds that iodide and bromide ions reduce the hydrogen yield although they do not react with solvated electrons, while acetone, which is a good electron scavenger, does not affect the yield at all. Therefore, he suggests excited water molecules as the species responsible for the "residual" hydrogen yield. It should be noted that relatively high concentrations of metal ions were used to observe these effects in Anbar's work. Usually, if a high solute concentration is required to reduce some product yield, it is indicative of interference with a track reaction. Thus, while Anbar's results may argue against e^-_{aq} being a precursor of hydrogen atoms it does not eliminate the possibility that atomic hydrogen is formed in a track reaction.

1.2.1 Summary

It is clear that our understanding of the radiolysis of

water, especially in neutral solution, is incomplete. It is agreed that the main reducing species in neutral, irradiated water is the solvated electron, with a yield of 2.7 ± 0.2 per 100 eV. The existence of an independent hydrogen atom yield at neutral pH is also fairly well-established. The oxidizing species formed is the hydroxyl radical, and molecular hydrogen and hydrogen peroxide are also produced. It is known that the radical yields vary with pH. However, with the re-determination of the yields in neutral solution, there is now agreement between the oxidizing and reducing equivalents in both acid and neutral solutions.

The question which is still unanswered is concerned with the source of the molecular products and the "residual" hydrogen atoms. Much of the controversy about this problem has been summarized by Anbar.[43] At the moment, there is evidence in favour of the "residual" hydrogen atoms coming from the spurs. Clearly what is needed to support the excited water molecule theories is positive identification of this unknown species. So far, this piece of information is lacking. If the work involving the transition metal ions could be repeated with low concentrations of ions, then this could be more seriously considered as an argument against "residual" hydrogen atoms from the tracks.

1.3 Radiation Biology

1.3.1 Introduction

Ideally, one would like to describe the end effects of radiation absorption by living matter in terms of the primary physical and chemical changes. There are a number of questions which must be answered

before it will be possible to reach a reasonably complete understanding of radiation-induced damage in living cells. Some of these include (i) what are the most important target macromolecules for damage? (ii) what kinds of structural defects are produced in these macromolecules? (iii) how do these defects arise following the absorption of radiation? (iv) how do they lead to the observed changes in living organisms?

Intense research is being carried out in both radiation chemistry and radiation biology to answer these questions. Some progress has been made on the first two questions in relatively simple chemical terms, a great deal of current work[47] is devoted to answering the third, while relatively little can be said about the fourth. One of the main difficulties is the time factor involved. Most physicochemical events concerned with the absorption of energy are completed within a few milliseconds under normal conditions, whereas the biochemical steps required to produce the observed damage may take several hours, days, or months. During this interval numerous variables may enter the picture, so that it is extremely difficult to attribute a certain injury (for example, cell death) to the absorption of a specific amount of radiation. Hence, there is still a tremendous gap between radiation chemistry and radiation biology in spite of the intense research being carried out in both fields.

Many research projects in both radiation chemistry and radiation biology are concerned with amino acids. There are several important reasons, in addition to purely theoretical ones, for studying the effects of radiation on amino acids in aqueous solutions.

Labelled amino acids are being widely used as tracers in biological research, so that it is important to know what transformations these compounds may suffer through autoradiolysis over long periods of time. Recently there has been an increased study of the radiation chemistry of proteins, including enzymes and hormones. In general, alteration of the secondary and tertiary structure rather than changes in the primary structure is observed. Such changes could result in reduced or completely altered biological activity, leading to dire consequences. The frequent failure to observe definite changes in the primary structure in a concentrated solution of a protein or within a living cell does not mean, however, that there is no change at all. It almost certainly implies that the analytical methods used are not subtle enough to measure the change. A thorough knowledge of the radiation chemistry of the amino acids participating in the protein structure is therefore a prerequisite to an understanding of the effects of radiation on the protein molecules themselves. Another aspect of radiation chemical studies includes the possible use of radiation-induced reactions of amino acids for synthetic purposes.

Of particular interest are the radiation chemical reactions of the amino acids containing sulfhydryl and disulfide groups. In addition to playing an important role in the biochemical processes of living organisms, many thioamino acids have been found to be active as protective or sensitizing agents. This aspect of their chemical behavior will be discussed in some detail.

1.3.2 Protection and sensitization

The concept of chemical protection arose from studies on purely chemical systems. Early work by Fricke[48] showed that in a solution containing two solutes the presence of one solute influenced the radiation chemistry of the other. These observations were attributed to a competition between the two solutes for the "activated water", the term used by early radiation chemists to describe the active species. If the addition of a relatively small amount of a substance, B, to a solution of another substance, A, results in a decrease in the radiation-induced decomposition of A, the process is called "protection". That is, the added compound, B, "protects" the original substance, A, from the effects of radiation. If the decomposition of A is enhanced by such a procedure, it is called "sensitization". Three basic types of protection can be distinguished, energy and charge transfer, radical scavenging, and repair:

(i) energy and charge transfer

Energy is transferred from an energy-rich species (ion or excited molecule) to the protector molecule which serves as an energy sink. Since either charge transfer or energy dissipation is involved, it is essentially a physical process at the molecular level. The result is to remove energy from a molecule which would otherwise dissociate or react, and thus to reduce the extent of decomposition. For example, the presence of benzene vapor is found to reduce the radiation-induced polymerization of acetylene.[49] The protective action is believed to be due to charge transfer

between acetylene ions and benzene:

$$C_2H_2^+ + C_6H_6 \longrightarrow C_2H_2 + C_6H_6^+ \qquad (27)$$

(ii) radical scavenging

Preferential radical scavenging by the more reactive solutes in an irradiated solution is often observed in radiation chemistry. This type of protection is particularly applicable when the substance to be preserved is present at very low concentrations and must be protected against indirect action rather than direct hits. By "direct hit" is meant the interaction of the compound in question with the ionizing radiation, leading to the formation of ions or excited species,

$$A \xrightarrow{\sim} A^+ + e^-$$

$$A \xrightarrow{\sim} A^*$$

Indirect action refers to interaction of the ionizing radiation with some other compound present, resulting in activated species, which, in turn, react with the compound in question and lead to the observed damage. The early work by Fricke[48] on the radiolysis of aqueous solutions is an example of protection due to radical scavenging. It is apparent that protection of sensitive biological materials in aqueous solutions by the addition of certain compounds is at least partly due to radical scavenging. Compounds which

are effective chemical protectors are often those which react readily with free radicals.

(iii) repair

A third type of protection, called repair, has been demonstrated by Alexander and Charlesby[50] in the radiolysis of polymers. A macromolecule, RH, is converted by radiation (either direct or indirect) to a free radical, R, which can then undergo a variety of reactions that lead to its inactivation - for example, crosslinking:

$$R + R \longrightarrow R\text{-}R \qquad (28)$$

or the formation of peroxy radicals:

$$R + O_2 \longrightarrow RO_2 \qquad (29)$$

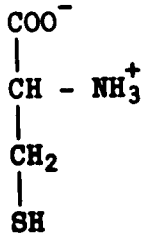
The protective compound may donate a hydrogen atom, thus restoring the "target" molecule to its original state:

$$R + \underset{\text{(protector)}}{PH} \longrightarrow RH + P \qquad (30)$$

before either of the above two reactions may occur.

The terms "protection" and "protective agent" were introduced into radiation biology in the early 1940's by Dale[51] and other workers[51] who discovered the ability of various sulfur compounds to inactivate the radicals generated in irradiated water, and by Barron[51] who observed the great sensitivity of thiols to the indirect action of ionizing radiation.

However, it was not until 1949 that chemical protection *in vivo* was discovered. At this time the protective action of cysteine (I), glutathione (II), and sodium cyanide were noted.[51,52] Since then



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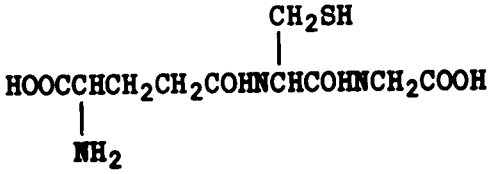
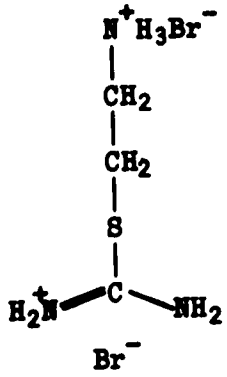


II

many substances have been studied for possible protective action. Probably the most important advances have been the discovery of cysteamine (III) and its S-guanido derivative, aminoethylisothiuronium bromide hydrobromide (AET) (IV).

III



IV

The terms "protection" and "sensitization" as applied to biological systems are usually restricted to mean a reduction and enhancement in the radiation response accomplished by procedures acting

before and during the irradiation. It is generally agreed that the term "restoration" should be used to denote a reduction in response accomplished by post-exposure procedures. This restrictive definition of the above terms seems valid since the effects brought about by pre- and post- treatments appear to be different, both in nature as well as degree.

Information concerning the mode of action of protecting or sensitizing compounds may be obtained by measuring the relationship between the radiation response and the amount of substance administered. Since many protective substances are toxic in high doses, the optimal protective effect which can be obtained will be a compromise between the toxicity and the protective action.

1.3.3 Protective compounds

Numerous chemical agents have been tested for protective action.[51,53] Many have shown small beneficial effects, while many others give little or no protection.[54] Of those which do possess protective ability, only a few are of any practical value. The remainder are too toxic when administered in the quantities required to provide protection. Nevertheless, the study of these compounds is still useful in furthering an understanding of radiation action at the molecular level.

The relatively high <u>in vitro</u> sensitivity of the thiol group to radiation was recognized by early workers in this field.[55] The -SH containing amino acid, cysteine, shows some protective action

when administered to a wide variety of organisms, and the tripeptide, glutathione, is also effective. Later work[55] has shown that the amine of cysteine, cysteamine, is also strongly protective. The sensitivity of certain thiols to the indirect action of ionizing radiation suggests that free radical scavenging ability and protective ability may be related.

From an investigation of a series of compounds related to 2-mercaptoethylamine, Doherty *et al*[54] concluded that there was a specific structure needed in order for a compound to exhibit protective properties. An amino group and a thiol (or disulfide) group separated by not more than three carbon atoms seemed to give maximal protection. A study of compounds related to AET[51] has also indicated the necessity of having two functional groups (guanido and thiol) separated by not more than three carbon atoms.

1.3.4 Mode of action of chemical protective agents

One of the most striking phenomena in the area of chemical protection is the pronounced *in vivo* specificity of certain compounds - especially the protective thiols. In animals only those thiols chemically related to cysteine - i.e. the cysteine-cysteamine group, are active. Many attempts have been made to explain the protective action of this group. In general they may be classified according to mode, namely those operative against direct action, those against indirect action, and repair.

(i) against direct action

Lea[56] has proposed that the major radiation damage is caused by direct hits of the ionizing particles on vital molecules. Although protection by chemical agents against such action is difficult to envisage, there is some experimental evidence in support of this theory. From studies involving radiation-induced electron spin resonance in proteins and other biological systems, Gordy et al[57] suggested that certain chemicals protect against direct hits by becoming temporarily bonded to the target molecules. Thus an unpaired electron or an electron hole caused by the interaction of the ionizing radiation with the target molecule could be passed onto the protector molecule, and eventually caught in a sulfur trap or some other trap in the side group.

(ii) against indirect action

Since water occupies about 60 - 70% of the mass of living matter,[58] the cell may be considered as an aqueous suspension of many different molecules. At least a portion of the radiation damage must occur by indirect action - that is, the ionizing radiation interacts with the water, and it is the subsequent reactions of the active species, produced from water, with the cellular constituents that result in radiation damage.

Numerous studies of the radiation chemistry of sulfhydryl compounds support their role as free radical scavengers.[59] Kumta et al[59] have envisaged the protein molecules in aqueous solution as being encompassed by molecules of the sulfhydryl protector. The thiol compounds thus would be readily accessible for reaction with the free

radicals produced in water. The chemical protector and the protein molecules would be in competition for the free radicals. If the thiols act as protective agents in this manner then they must be able to convert these radicals to less active radicals - that is, the new radicals must be less likely to attack vital cellular constituents than are the active species in water. In biological systems, other free radicals from the cells may be formed by direct hits, and interaction of the protective agent with these secondary free radicals must also be considered.

Unfortunately, very little quantitative data on the radical-capturing ability of the numerous protective compounds are available.

Eldjarn and Pihl[51] have proposed a mechanism which is designed to account for protection against both direct and indirect action. They contend that cysteamine protects by forming a mixed disulfide linkage with the thiol or disulfide groups of the protein. The following simplified equation illustrates the process:

$$\underset{\substack{\text{(protector}\\\text{thiol group)}}}{PSH} + \underset{\substack{\text{(protein}\\\text{thiol group)}}}{HSR'} \rightleftharpoons \underset{\text{(mixed disulfide)}}{PSSR'} \quad (31)$$

Protection of the protein may occur in two ways:

(i) against direct action, since the protein disulfide is more resistent to direct radiation than the original protein.

(ii) against indirect action, since free radical attack on the disulfide link results in destruction of the protector thiol

group and the regeneration of the protein thiol group. Eventually, by metabolic processes, the -SP group is removed and the protein regenerated.

The mixed disulfide mechanism is now supported by a considerable body of evidence. Simple disulfides, for example, have been found to be highly susceptible to the indirect action of ionizing radiation. In particular, the radiation-induced disappearance of cystine has been studied.[55] The degradation products have indicated that the radiation chemical reactions all took place at the sulfur atoms. Further evidence for sulfur participation has been found from studies of protein disulfides,[55] in which the main result of the indirect action is oxidation of part of the sulfur with a significant fraction being reduced to thiols.

Many studies of the ability of thiols and disulfides to enter into mixed disulfide formation with cysteine, cystine, and oxidized and reduced glutathione have been carried out.[51] These compounds were chosen as prototypes of target molecules, since most of the cellular thiol and disulfide groups are contributed by the cysteine and cystine residues of peptides and proteins. It was found that those thiols which exhibit a strong protective activity react rapidly with cystine, whereas those with little or no activity in general react slowly with cystine. The observed reaction rates would thus appear to support the mixed disulfide mechanism.

The immediate protection offered *in vivo* by the cysteine-cysteamine group and the limited duration of this protection would seem to imply that the binding of the protective agents to the target molecules must be of a temporary nature. This is in agreement with the energy transfer theory proposed by Gordy.[57]

From the data available it is evident that disulfide groups occupy a central position in the mechanism of protection - against both direct and indirect action. The observation that a number of thiols are protective *in vitro* but are not protective *in vivo* may be explained by assuming that these compounds are unable to form mixed disulfides with the target molecules. However, although ability of thiols to form mixed disulfides is in qualitative agreement with their sensitivity to radiation, it does not reflect the direction of the effect nor give any indication of their relative protective activities. Furthermore, this theory cannot explain the protective ability of compounds which do not contain any thiol groups - notably those containing aromatic or indole rings. Many of these compounds are equally effective as or even more effective than thiols in energy-transfer processes.

(iii) repair

Deoxyribonucleic acid (DNA) and its protein constituents have often been considered as likely sites for the initiation of radiation damage in cells. One of the most striking effects of X- or γ-radiation is the crosslinking of the DNA molecule. Ormerod and Alexander[60] have studied the crosslinking of DNA in an oxygen-free system. In the presence of cysteamine they found that crosslinking was decreased. They

suggested that this protection of the DNA from direct hits was due to repair by a hydrogen transfer process. The ESR spectrum of the irradiated DNA-cysteamine mixture was found to be similar to the spectrum obtained from irradiated cystine and was attributed to an -S radical formed from the cysteamine. However, the intensity of the spectrum was far greater than that which would have been expected as a result of direct hits on cysteamine. The authors suggested the following reaction

$$R + NH_2CH_2CH_2SH \longrightarrow RH + NH_2CH_2CH_2S\cdot \qquad (32)$$

where R refers to a damaged DNA molecule. In this way radiation damage in DNA is repaired, and radical combination leading to crosslinking is prevented. Unfortunately, the precision of the data was not great enough to make it possible to obtain information regarding the exact nature of the above reaction. The repair mechanism can however account for the marked increase in protection by cysteamine in the absence of oxygen. A competition between -SH groups and oxygen for the radiation-produced radicals should reduce the protection offered by cysteamine in the presence of oxygen.

It is impossible, confronted with the limited amount of data available and the complex structural system of the living cell, to conclude that any one mechanism of protection is correct. Since the cell is not a homogeneous solution,[58] the effects of the radiation could vary from one part of the cell to another, so that different mechanisms may be operative in different localities. Thus, all of the proposed

theories may be partly correct.

1.3.5 Summary

Within the last few decades many hypotheses have been proposed to explain quantitatively the effects of ionizing radiation on simple biological systems.[61] The most important of these are the "direct action" and the "indirect action" hypotheses. The latter includes all attempts that attach primary importance to the reactive intermediates generated by irradiation of the surrounding water, or to organic peroxides produced directly or indirectly. Often the results of classical photochemistry and radiation chemistry have been transposed without due regard for the differences that obviously exist between the highly purified gaseous and liquid systems used by chemists and the complex, highly organized material that exists in the cell studied by the biologists. This danger must be recognized. However, there are several still unanswered questions upon which radiation chemical studies may cast some light. One of these involves the nature of the species responsible for radiation damage in enzymes and nucleic acids. Some correlation has been achieved between competition kinetic experiments and electron spin resonance studies in the solid state. More precise techniques in both fields are developing and should provide additional quantitative data on which to base radiolytic mechanisms. Another question concerns the importance of reactions in pure water or dilute, aqueous solutions when applied to systems containing 20% solute such as a living cell. The reactions and rates of reactions of the reactive intermediates

produced in irradiated water may be completely different in relatively concentrated solutions.[61] It is well known that the yield of molecular products formed in irradiated aqueous solutions decreases as the solute concentration is increased. At the same time the yield of radical products increases.[62] These changes are attributed to penetration of the solute molecules into the tracks, resulting in interference with the normal track reactions and the subsequent diffusion of the radicals. Nonetheless, studies of the radical species and their reaction times in pure water and dilute solutions serve as guides to the understanding of the effects of radiation in dilute solutions of enzymes and nucleic acids, and, hopefully, of the effects in the heterogeneous, concentrated, structural matrix that is the cell.

Eldjarn and Pihl[51] noted that the thiols and disulfides which are chemically related to cysteine and cysteamine "constitute the group of protective agents which has been most extensively used *in vivo* and which seems to offer greatest promise". In addition the cysteine molecule is itself an important constituent of certain proteins and enzymes. For these reasons the present study has been concentrated on the fundamental radiation chemistry of dilute aqueous solutions of cysteine.

1.4 Previous Studies of Cysteine and Scope of Present Work

There are indications from the literature that reactions of cysteine with the various free radicals produced from irradiated water are rather complex. The variety of products and the complexity of product

analysis have made it very difficult to reach any conclusions regarding the details of the radiolytic mechanism. This is especially true in aerated solutions.

1.4.1 Aerated solutions

The radiolysis of dilute aqueous solutions of a number of thiols including cysteine[63,64,65] has been studied by various workers who have shown that in oxygenated systems short chain reactions occur, yielding the corresponding disulfides as the major products.[66] Swallow[63] suggested the following mechanism:

$$H_2O \longrightarrow G^M_{H_2}H_2 + G^M_{H_2O_2}H_2O_2 + G_H H + G_{OH}OH \quad (1)$$

$$RSH + OH \longrightarrow H_2O + RS \quad (33)$$

$$H + O_2 \longrightarrow HO_2 \quad (34)$$

$$RSH + HO_2 \longrightarrow RS + H_2O_2 \quad (35)$$

$$RS + RSH \longrightarrow H + RSSR \quad (36)$$

Reactions (34), (35), and (36) propagate the chain.

It was shown from studies of reactions of hydrogen atoms with cysteine[68] that the following reaction occurs readily:

$$RSH + H \longrightarrow H_2 + RS \quad (37)$$

Thus, in the absence of oxygen, a chain consisting of reactions (36) and (37) should take place. The fact that it does not[63,64] and that reaction (36) is endothermic to the extent of about 17 kcals per mole makes this mechanism unlikely.[66]

Whitcher *et al*[64] studied the radiolysis of both oxygenated

and deoxygenated dilute, aqueous cysteine solutions. The results obtained were consistent with oxidation by hydroxyl radicals and hydrogen sulfide or hydrogen formation by hydrogen atoms, but in the absence of a chain reaction the high yields could not be explained (e.g. G(-cysteine) = 15.4 and $G(H_2O_2)$ = 6.2 in neutral solution). The authors noted that cysteine and hydrogen peroxide were stable together for several hours in acid solution. Their results from deaerated solutions will be discussed later.

Further studies on aerated solutions were carried out by Markakis and Tappel.[65] In addition to cystine and hydrogen sulfide other products such as free sulfur (G ∿ 1), sulfate (G = 2.3), ammonia (G = 1.9), and alanine (G = 3.8) were found. Packer[66] investigated the radiolysis of aqueous solutions of hydrogen sulfide and of cysteine, in the absence and presence of oxygen. The large G-values obtained in oxygenated solutions of hydrogen sulfide for sulfur and sulfate formation suggested a short chain mechanism:

$$HS + O_2 \longrightarrow HSO_2\cdot \qquad (38)$$

$$HSO_2\cdot + H_2S \xrightarrow{\text{slow}} HSO_2H + HS \qquad (39)$$

$$2\ HS \longrightarrow H_2S + S \qquad (40)$$

It was suggested that a similar mechanism is operative in radiolytic thiol oxidation:

$$RS + O_2 \longrightarrow RSO_2\cdot \qquad (41)$$

$$RSO_2\cdot + RSH \longrightarrow RSO_2H + RS \qquad (42)$$

It is noteworthy that $G(H_2O_2)$ in the presence of oxygen is approximately $G_H + G_{H_2O_2}$, showing that reaction (35) occurs and that cysteine and hydrogen peroxide do not react at pH 4. This is of importance in the present project.

1.4.2 Deaerated solutions

Dale and Davies[67] irradiated cysteine solutions with X-rays (dose $\sim 2 \times 10^{18}$ eV/gm) and determined the yield of hydrogen sulfide liberated. It was observed that in acid solutions hydrogen sulfide formation is very small. For 6×10^{-4} M cysteine solution, $G(H_2S)$ is less than 0.1, reaches a maximum at pH 6.5, and declines to a very small amount in alkaline solution. They concluded that in addition to oxidation of the thiol form to the disulfide, a reaction which is chemically reversible, there are irreversible reactions leading to the liberation of hydrogen sulfide. The production of hydrogen sulfide may be important from the biological point of view since its occurrence within the cell is likely to have a toxic effect. These authors found no ammonia with doses of $\sim 6 \times 10^{18}$ eV/gm at pHs 2, 4, and 6, indicating that deamination, which is important with other amino acids,[55] does not occur appreciably with cysteine.

The results obtained by Whitcher et al[64] in deaerated solutions indicated that the yields of both hydrogen peroxide and hydrogen sulfide were greatly reduced upon removal of oxygen, $G(H_2O_2) = 0.77$ and $G(H_2S) = 2.5$ in neutral solution. Although measurements were not made below pH 4, it would appear that below pH 8, $G(H_2S)$ is appreciable

- about 2.5 for 4×10^{-4} M cysteine solution. The authors noted that in acid solution the yield of hydrogen peroxide was not measurable, although it was not zero.

A study of reactions between hydrogen atoms generated in a discharge tube and an air-free aqueous solution of cysteine was carried out by Littman, Carr, and Brady.[68] They found that in the pH region 0 - 6, cystine was the major product, and hydrogen sulfide was formed in small amounts. They postulated the following reactions involving hydrogen atoms in acid medium:

$$H + RSH \longrightarrow H_2 + RS \qquad (37)$$

$$H + RSH \longrightarrow H_2S + R \qquad (43)$$

RSH refers to cysteine. Cystine would be formed from dimerization of the thiyl radicals. Since cystine was the major product in this pH range, they suggested that reaction (37) was favoured over reaction (43). The above reactions are of concern in the present study since these experiments were also carried out in the pH range 0 - 7. As a point of interest it might be noted that above pH 8 hydrogen sulfide was the major product. Since the sulfhydryl group is ionized at $\sim$ pH 8, the authors suggested hydrogen atom attack on the ionized form of cysteine to account for hydrogen sulfide formation:

$$H + RS^- \longrightarrow SH^- + R \qquad (44)$$

$$SH^- + H^+ \longrightarrow H_2S \qquad (45)$$

Littman *et al*[68] found no indication of deamination.

Markakis and Tappel[65] investigated the γ-radiolysis of aqueous solutions of cysteine and cystine over a wide dose range. They agreed with Whitcher *et al*[64] that in the acid to neutral pH region, the amount of hydrogen sulfide formed is appreciable (G = 2.9 for aqueous 0.1 M cysteine solutions). An equal amount of alanine was found. They also reported a sizable yield of cystine (G = 4.4), and a very small amount of ammonia (G = 0.5) at a dose of 6×10^{19} eV/gm. The following sequence of reactions was suggested to account for the similarities in the hydrogen sulfide and alanine yields:

$$RSH + H \longrightarrow R + H_2S \quad (43)$$

$$R + H \longrightarrow RH \quad (46)$$

$$R + RSH \longrightarrow RH + RS \quad (47)$$

$$RSH + H \longrightarrow RH + SH \quad (48)$$

$$SH + H \longrightarrow H_2S \quad (49)$$

Since the major product is cystine, reactions of the intermediates formed from water with cysteine were considered:

$$RSH + OH \longrightarrow H_2O + RS \quad (33)$$

$$RSH + H \longrightarrow H_2 + RS \quad (37)$$

$$RSH + HO_2 \longrightarrow H_2O_2 + RS \quad (50)$$

$$2\ RSH + H_2O_2 \longrightarrow 2\ RS + 2\ H_2O \quad (51)$$

$$2\ RS \longrightarrow RSSR \quad (52)$$

It was shown by Whitcher et al[64] and later by Packer[66] that the reaction of cysteine and hydrogen peroxide in acid solution is very slow.

It is apparent that the large hydrogen sulfide yield found in the γ-radiolysis of aqueous cysteine solutions cannot be accounted for on the basis of hydrogen atom attack as shown in reaction (43), since, as it was shown by previous workers,[68] in this pH range the hydrogen sulfide yield from hydrogen atom attack is small. Therefore, it must be concluded that there is some other species which is reacting with cysteine to give hydrogen sulfide.

Let us consider some of the possible reactions involving the reactive intermediates from irradiated water. Since it is now known that the reducing species in irradiated water exists in two forms, it is necessary to include possible modes of attack of both hydrogen atoms and solvated electrons on cysteine. Hydrogen and hydrogen sulfide could result from hydrogen atom attack, and, as indicated by the discharge tube work,[68] hydrogen would be the main product of this reaction. However, no hydrogen yields have been reported in the earlier studies involving ionizing radiation. Detailed studies of hydrogen production are obviously required.

Attack of the solvated electron on the ammonium group might be expected:

$$e^-_{aq} + RSH \longrightarrow NH_3 + R' \qquad (53)$$

but earlier workers had found very little ammonia.[65,67] Thus it would appear that this reaction is of secondary importance.

A reaction of the solvated electron with cysteine to give hydrogen sulfide, viz:

$$e^-_{aq} + RSH \longrightarrow R + SH^- \quad (54)$$

$$SH^- + H^+ \longrightarrow H_2S \quad (45)$$

is an obvious possibility. Reaction (54) would have to compete with reaction (55)

$$e^-_{aq} + H^+ \longrightarrow H \quad (55)$$

which, in acid solution, is assumed to convert the solvated electrons to hydrogen atoms. Such a competition would be analogous to that previously observed with monochloroacetic acid,[20,21] where the reaction

$$e^-_{aq} + ClCH_2COOH \longrightarrow Cl^- + \cdot CH_2COOH \quad (56)$$

competes with reaction (55).

The known yields of solvated electrons produced in irradiated water suggest that reaction (54) may be expected to give a hydrogen sulfide yield of about 2.5 - 2.8 molecules per 100 eV. Markakis and Tappel[65] reported $G(H_2S) = 2.9$ in 0.1 M cysteine solution at pH 1.6. They reported a similar yield of alanine which would be anticipated if reaction (54) is followed by reaction (47):

$$R + RSH \longrightarrow RH + RS \quad (47)$$

From the reactions of hydrogen atoms and hydroxyl radicals with cysteine

which have been proposed by earlier workers[65,68] the expected yield of cystine may be calculated to be approximately 3.3 in acid solutions (for more details on this determination the reader is referred to p.88 of the Discussion). Markakis et al[65] observed 4.4 molecules per 100 eV.

1.4.3 Scope of present study

It is clear from the above review of irradiated cysteine solutions that there is some agreement between different workers on the quantitative yields of various products. Their results are summarized in Table I. However, the existing information on the radiolysis of cysteine was by no means complete at the time this study was initiated. There was an obvious lack of data on hydrogen formation and quantitative yields of many of the other products had not been studied in detail. For these reasons a study of the products of radiolysis of cysteine under deaerated conditions and over a range of doses, concentrations and values of pH was undertaken. One specific aim was to determine whether reaction (54) was a plausible mode of hydrogen sulfide formation and a satisfactory explanation for the different pH behaviour of the hydrogen sulfide yields from the radiolysis and the hydrogen atom reactions with cysteine.

In order to elucidate some of the details of the radiolytic mechanism, product yields independent of solute concentration and dose should be found. To this end a complete quantitative product analysis was planned.

For reasons which will become apparent parallel studies of the radiolysis of aqueous solutions of 2-mercaptoethanol and methyl mercaptan were also carried out in this laboratory.

TABLE I

Deaerated solutions

[Cysteine]	pH	Dose (eV/gm)	G(-RSH)	G(Cystine)	$G(H_2S)$	G(Alanine)	$G(H_2O_2)$	$G(NH_3)$	G(S)	$G(SO_4^{=})$	Reference
6×10^{-4}M	acid	2×10^{18}	-	-	<0.1	-	-	-	-	-	(67)
6×10^{-3}	acid	2×10^{18}	-	-	0.5	-	-	-	-	-	(67)
3.2×10^{-2}	6	6×10^{17}	-	-	1.3	-	-	-	-	-	(67)
4×10^{-4}	6	1.25×10^{18}	-	-	2.47	-	0.77	-	-	-	(64)
4×10^{-4}	1	1.25×10^{18}	-	-	small	-	-	-	-	-	(64)
10^{-1}	1.5	6×10^{19}	13.0	4.4	2.9	2.9	-	0.5	-	-	(65)
4.4×10^{-3}	4	8×10^{19}	6.2	-	-	-	0.6	-	-	-	(66)

Aerated solutions

4×10^{-4}	1	1.25×10^{18}	9.2	-	0.6	-	7.1	-	-	-	(63)
4×10^{-4}	4	1.25×10^{18}	6.2	-	0.6	-	4.6	-	-	-	(64)
4×10^{-4}	7	1.25×10^{18}	15.4	-	0.6	-	6.2	-	-	-	(64)
10^{-1}	1.5	6.2×10^{19}	33.5	13.0	2.52	3.76	-	1.93	0.96	2.31	(65)
4.4×10^{-3}	4	7×10^{19}	13	-	-	-	4.4	-	-	-	(66)

2. EXPERIMENTAL

2.1 Apparatus

A mercury-free system (see Figure 1) was constructed inside a fume cupboard for the preparation of aqueous solutions of cysteine. The same vacuum line was used for the preparation of solutions of 2-mercaptoethanol and methyl mercaptan, and for the storage of methyl mercaptan. A Duo-Seal vacuum forepump and an oil diffusion pump manufactured by the Consolidated Vacuum Corporation provided a vacuum of 10^{-5} torr. Bulbs of various sizes were attached to the manifold for gas storage and bulb-to-bulb distillations. Pressure measurements in the range 1 to 700 mm. were made with a stainless steel differential diaphragm gauge. One side of this was connected to an auxilliary vacuum line with a mercury manometer. A discharge vacuum gauge was also incorporated into the system.

The hydrogen analysis system is shown in Figure 2. It was equipped with a Duo-Seal forepump and an all-glass two-stage mercury diffusion pump. A palladium thimble was used to remove hydrogen. The volume of the McLeod gauge and associated dead space (E in Figure 2) was 394 cubic centimeters. The liquid nitrogen trap B prevented the distillation of mercury from the Toepler pump D into the sample through C.

A typical irradiation cell is shown in Figure 3a. A standard taper 10/30 inner joint was attached to the cell to facilitate connection to the vacuum system. In order to obtain thorough degassing by shaking the solution under vacuum, a piece of flexible Tygon tubing was connected to the capillary and standard taper joint A, as shown in Figure 3a. After the sample had been degassed and frozen 7 times, the capillary was collapsed, sealing the cell under vacuum. When hydrogen yields were to be determined,

Figure 1

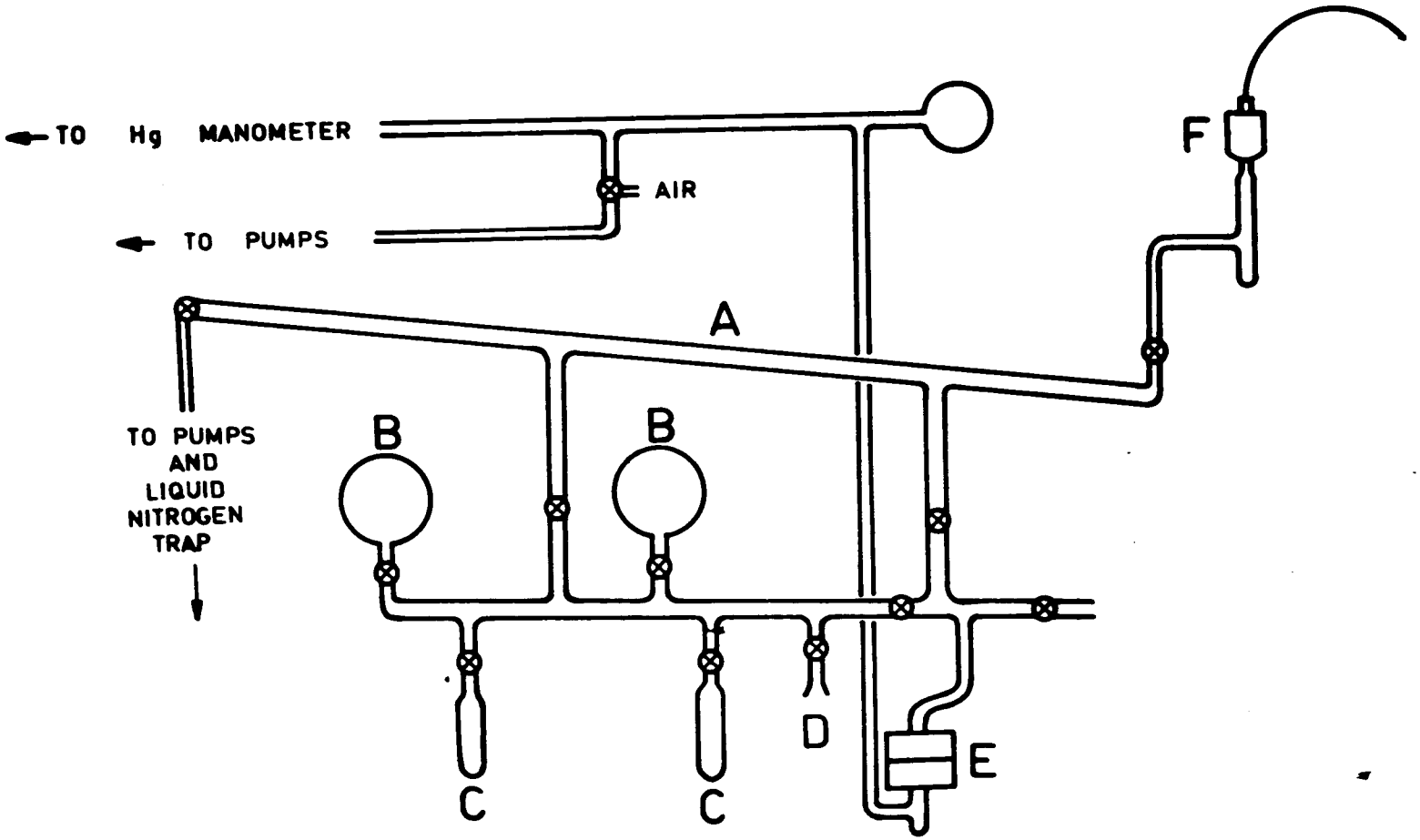

TO Hg MANOMETER
AIR
TO PUMPS
A
F
TO PUMPS AND LIQUID NITROGEN TRAP
B
B
C
C
D
E

Figure 1

Sample Preparation Line

A -- Main manifold

B -- Storage bulb

C -- 20 cc bulb

D -- Sample connection

E -- Diaphragm gauge

F -- High vacuum gauge head

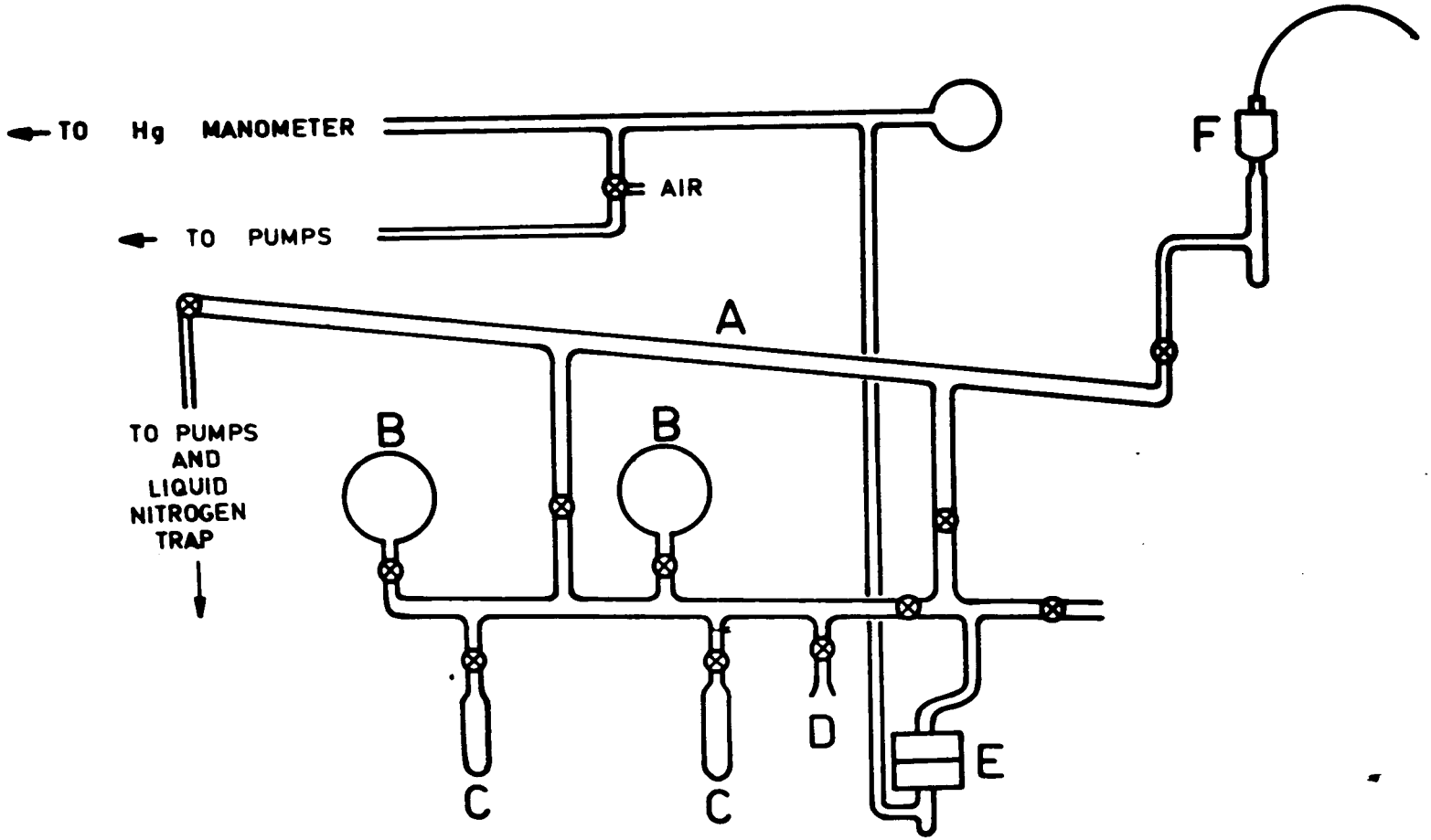

TO Hg MANOMETER
AIR
TO PUMPS
F
A
TO PUMPS AND LIQUID NITROGEN TRAP
B
B
C
C
D
E

Figure 2

Figure 2

Gas Analysis Line

A -- Main manifold

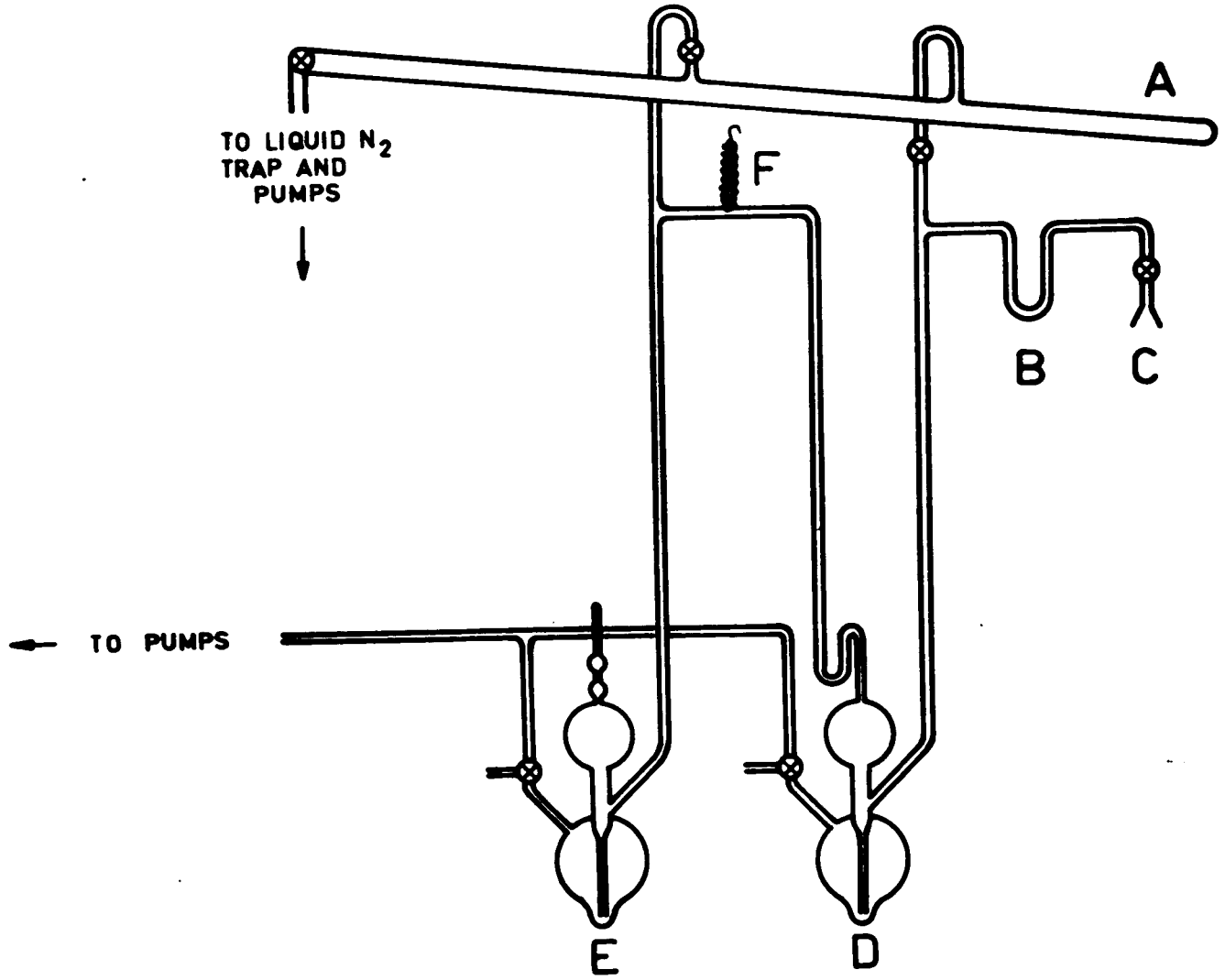
B -- Liquid nitrogen trap

C -- Sample inlet

D -- Toepler pump

E -- McLeod gauge

F -- Palladium thimble


TO LIQUID N_2
TRAP AND
PUMPS
A
F
B
C
TO PUMPS
E
D

Figures 3a and 3b

Figure 3a

Irradiation Cell

A -- Standard taper 10/30 inner joint

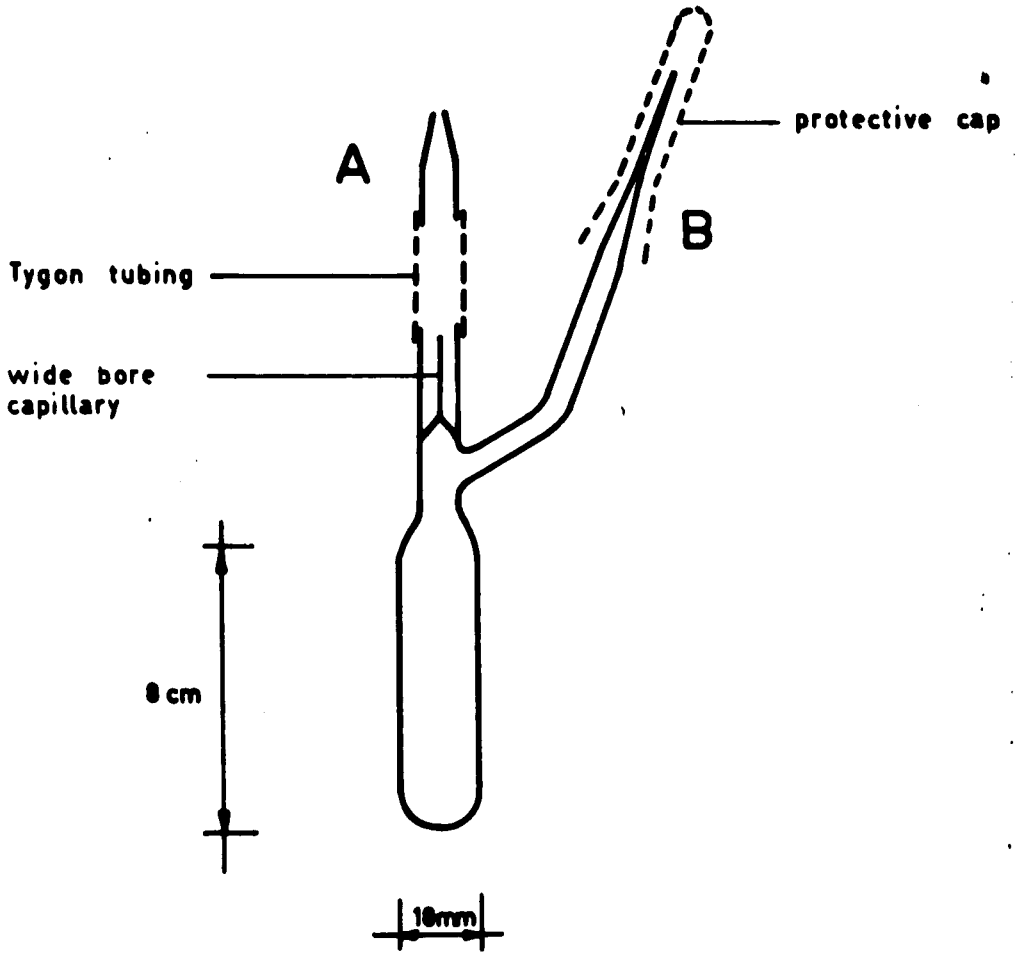
B -- Standard taper 10/30 inner joint with tip

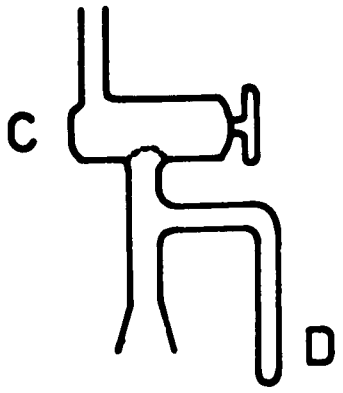
Figure 3b

Adapter Apparatus

C -- Offset sidearm stopcock

D -- Small sidearm


protective cap
A
B
Tygon tubing
wide bore
capillary
8 cm
10mm


C
D

the end of joint B was drawn to a fine tip. It fitted into the barrel of the stopcock C of the adapter apparatus shown in Figure 3b. When the entire assembly was connected to the hydrogen analysis line, this cell could be opened under vacuum simply by turning stopcock C and breaking the tip. The small sidearm, D, was constructed for the purpose of bulb-to-bulb distillation of a small portion of the irradiated solution.

When the irradiated solutions were to be analyzed spectrophotometrically for cystine, cells such as the one depicted in Figure 4 were used. Since cysteine is easily oxidized to cystine in neutral solutions, it was essential that measurements be made without opening the cell and exposing the contents to the atmosphere. A quartz spectrophotometric cell was attached to the irradiation cell and arranged in such a way that it could be protected from the ionizing radiation by a lead brick. The analysis was carried out using a Beckman DU Spectrophotometer, model 2400.

Alanine analyses were carried out with a Technicon Amino Acid Analyzer. The column consisted of a microspherical sulfonic acid-type cation exchange resin of high exchange capacity ("Chromobead type C-2"). Two colorimeters were used in series, one with a 15 mm. tubular light path and a 570 mμ interference filter, and another with a 15 mm. tubular light path and 440 mμ interference filter. The colorimeters were of the dual beam type, utilizing two separate photocells, one as reference and the other for the sample. A chart recorder was used to record the optical density.

The Co^{60} source consisted of approximately 100 curies

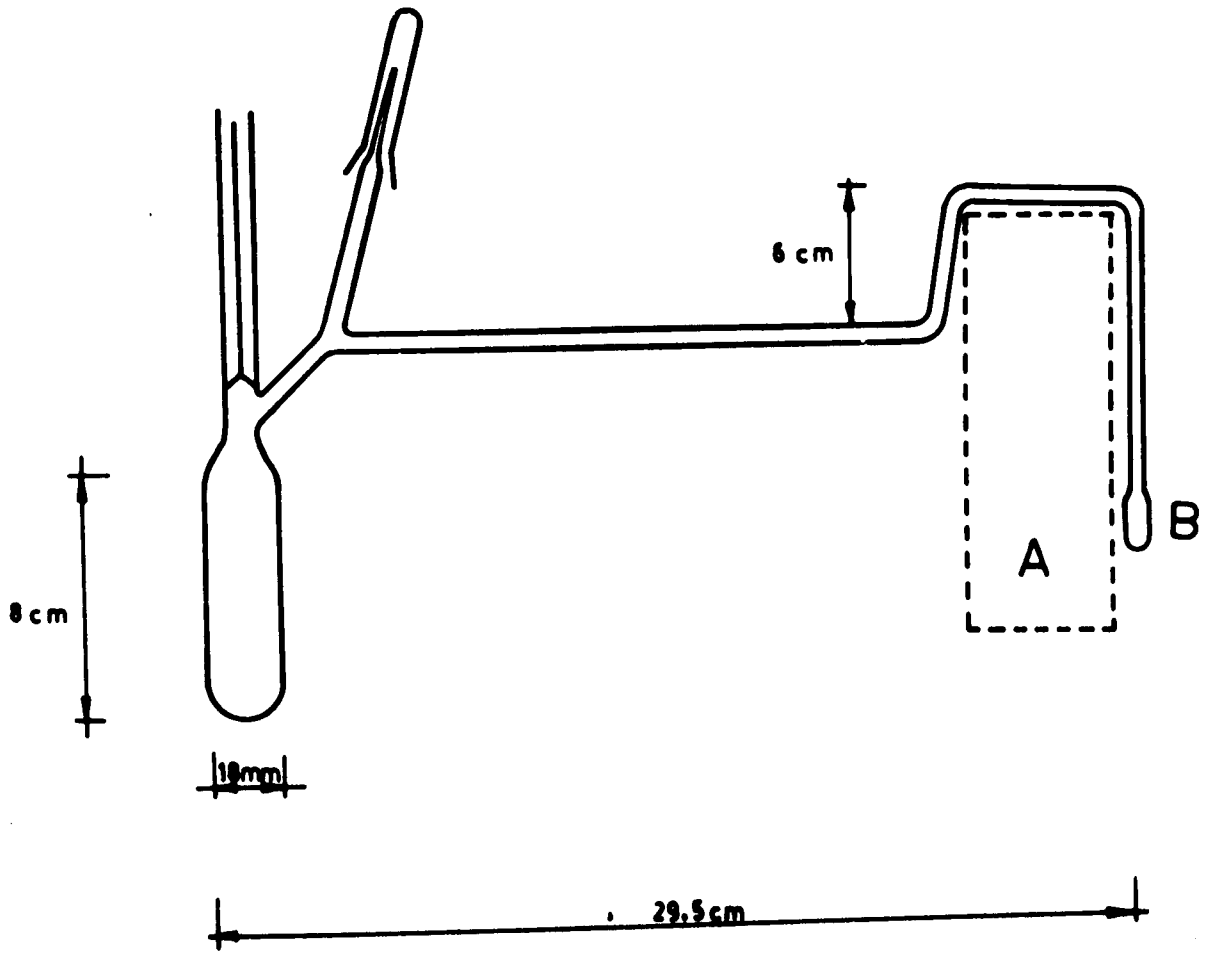
Figure 4

Figure 4

Modified Irradiation Cell

A -- Lead brick

B -- Quartz spectrophotometric cell


6 cm
A
B
8 cm
18mm
29,5cm

which could be lowered into a concrete irradiation cave by means of a pulley system. The irradiation cells were inserted into the cave by means of a trolley which ran on fixed rails. To ensure reproducibility of sample position, an aluminum cell holder was bolted to the trolley floor.

2.2 Materials

Methyl mercaptan was obtained from the Matheson Company as a compressed gas and was used as received without further purification. Cysteine and cystine were both purchased from the Mann Research Laboratories, New York, and used as received. Alanine and 2-mercaptoethanol were supplied by Matheson, Coleman and Bell. The mercaptoethanol was redistilled under vacuum, b.p. = 55°C at 10 - 13 mm. Hg. Acetone, which was used in the competition kinetics studies, was Shawinigan Chemicals reagent grade, density = 0.7857 gm/ml at 25°C. Ferrous ammonium sulfate, ammonium molybdate, and potassium nitrate were all Mallinckrodt reagent grade. Zinc acetate, sodium acetate, ferric chloride, and phosphoric acid were purchased as reagent grade chemicals from the Fisher Scientific Company. A reagent grade sample of p-aminodimethylaniline sulfate was obtained from Eastman Organic Chemicals for use in hydrogen sulfide analysis. It was used as received.

In order to obtain "double distilled water", either ion-free water or "laboratory distilled water" was distilled from an alkaline permanganate solution in an all-glass still. The purified water thus obtained was stored in Pyrex Brand glass flasks with ground-glass stoppers.

All reagents and samples were prepared with this water.

2.3 Dosimetry

The Fricke dosimeter was used for dose rate determinations. The solution was prepared according to the directions of Weiss[4] using reagent grade ferrous ammonium sulfate, hydrochloric acid and sulfuric acid. The water was "double distilled". Dosimetry tubes of the same dimensions as the irradiation cells were first cleaned with permanganic acid, then rinsed three times each with tap water, distilled water, "double distilled water", and finally with the dosimetry solution.

The solutions were irradiated for periods of 30, 60 and 90 minutes and the resulting concentrations of Fe(III) were determined at 224 mμ[5] with a Beckman DU Spectrophotometer. At this wavelength, the molar extinction coefficient for Fe^{+++} in 0.8 N sulfuric acid at 25°C was taken as 4500.[5] The dose rate from the dosimetry solution is given by the following expression:

$$\text{(i)}\quad \text{Dose rate} = \frac{\Delta OD}{\Delta t} \times \frac{N}{\varepsilon} \times \frac{100}{G(Fe^{+++})} \times \frac{1}{1000} \quad \text{eV/ml/time}$$

where $\frac{\Delta OD}{\Delta t}$ is the slope of the optical density versus time plot of the irradiated Fricke solution, N is Avogadro's number, ε is the appropriate molar extinction coefficient of Fe^{+++}, and $G(Fe^{+++})$ is 15.6.[1] Since the absorbed dose is a function of the electron density of the absorbing media, the dose rate calculated from expression (i) is applicable only to solutions with the same electron density as the 0.8 N sulfuric acid solution. The dose rates used in this investigation have been corrected for the

ratio of electron densities between the dosimetry solution and the sample to be irradiated. Thus expression (ii) represents the dose rate for a cysteine solution:

$$\text{(ii)}\quad (\text{Dose rate})_{\text{cysteine}} = (\text{Dose rate})_{\text{Fricke}} \times \frac{\rho_{\text{cysteine}}}{\rho_{\text{Fricke}}} \times \frac{\text{e.d.}_{\text{cysteine}}}{\text{e.d.}_{\text{Fricke}}} \quad (\text{eV/ml/sec})$$

In the above expression, e.d. refers to the electron density of the solution in question, while ρ refers to the density. A typical plot of optical density versus time as obtained for three different positions in the irradiation cell holder is shown in Figure 5. Since the Co^{60} source decays by approximately 1% per month, the dose rates were recalculated every month. Periodically they were redetermined experimentally as a check. The experimental determinations agreed well with the predicted decay rate.

2.4 Procedures

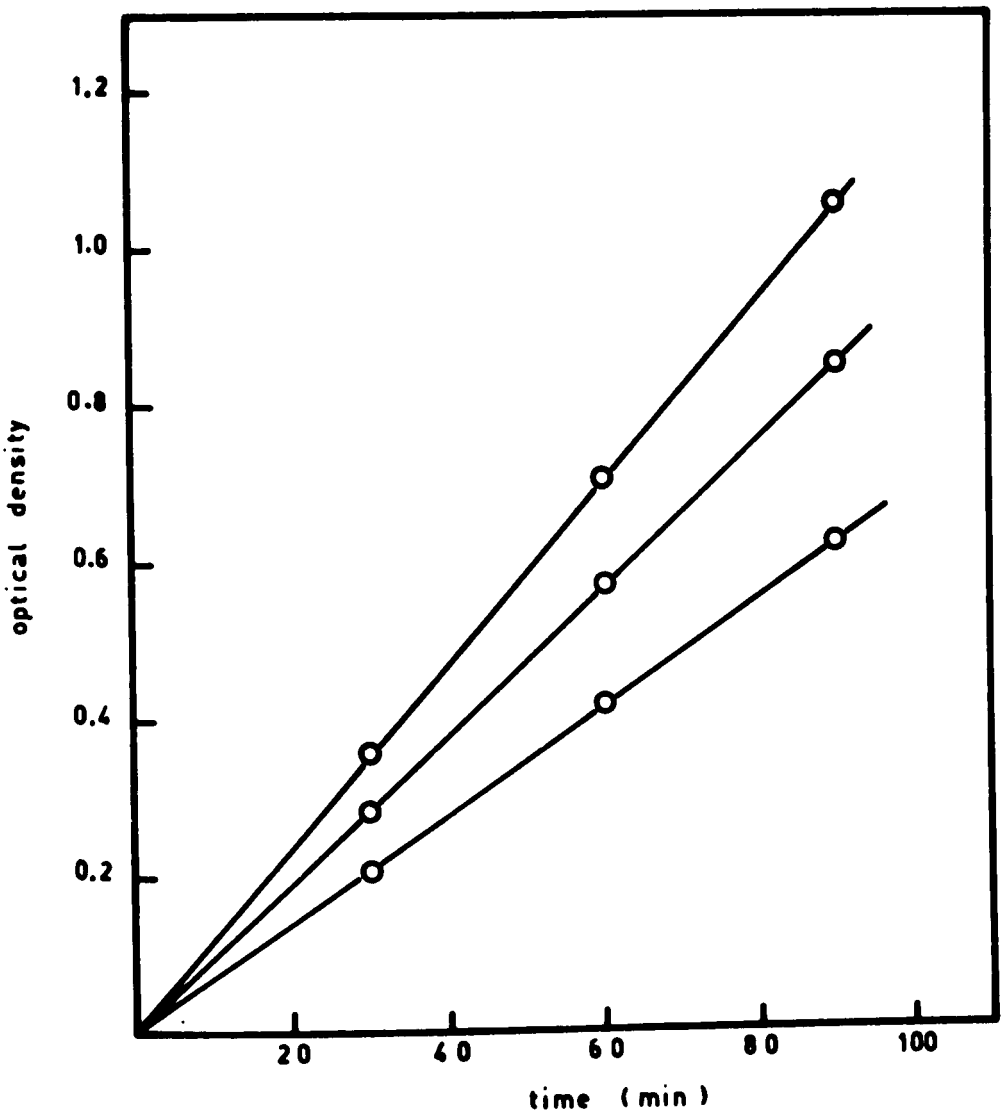
2.4.1 Sample preparation

All methyl mercaptan samples were measured in the gas phase using standard volumes and the diaphragm gauge described in the Apparatus section. After measurements, the methyl mercaptan was frozen at liquid nitrogen temperatures (-196°C) into the irradiation cell, which contained a known amount of thoroughly degassed aqueous acid solution. The capillary was then collapsed, sealing the sample under vacuum. The acid was melted and shaken at ambient temperature to equilibrate the

Figure 5

Figure 5

Optical Density as a Function of Time for Dosimetry Solution


1.2
1.0
0.8
0.6
0.4
0.2
optical density
20
40
60
80
100
time (min)

mercaptan in the vapour and solution phases. The concentration of mercaptan in the solution was calculated using the solubility coefficient $(3.7 \pm 0.3) \times 10^{-3}$ M/l/cm. of Hg pressure of mercaptan above the solution, which had been previously determined for the pH range 0 - 6 and the pressure range 15 - 45 cm. of Hg.

Solutions of cysteine in perchloric acid, sulfuric acid or "double distilled water" were purged for 30 minutes with purified nitrogen to remove dissolved oxygen and carbon dioxide. They were then degassed by a procedure of successive freezing, pumping, and thawing, using first a slurry of dry ice and ethanol (-79°C) and then liquid nitrogen (-196°C). The samples were sealed off under vacuum. The pH of the solution was checked before and after irradiation whenever possible. The volume chosen for irradiation was five milliliters.

Solutions of 2-mercaptoethanol were prepared in "double distilled water" or acid solution which had been previously purged with purified nitrogen. They were then degassed and sealed off under vacuum in the same manner as the cysteine solutions.

2.4.2 Analyses

After radiolysis, the irradiation cells were attached to a gas analysis vacuum line (Figure 2) via the adapter apparatus shown in Figure 3b. The sample was frozen at -196°C, and the tip (B in Figure 3a) broken. The non-condensible gases were transferred by means of a Toepler pump to a McLeod gauge and the pressure measured. The hydrogen content of the sample was determined by heating the palladium thimble and measuring

the decrease in pressure when all the hydrogen had escaped. A small fraction of the hydrogen was always occluded by the solidified sample in the irradiation cell. This was released in the following way. Stopcock C was closed and the solution melted. A small portion of it was then distilled into the sidearm of the adapter apparatus (D in Figure 3b). During this procedure the escape of vapour bubbles facilitated the release of hydrogen. The solution was then frozen and this second quantity of hydrogen pumped over and measured. From the sum of the two yields the G-value for hydrogen formation was calculated. Further attempts to obtain a third quantity of hydrogen by repeating the above procedure indicated that essentially all of the measurable hydrogen had been collected in the previous attempts.

Yields of hydrogen sulfide in cysteine and mercaptoethanol solutions were measured colorimetrically by means of the molybdate reagent.[73] The tips of the sealed vessels containing 5 mls of irradiated solution were broken under the surface of a mixture of 5 mls of 5% zinc acetate and 5 mls of 1% sodium acetate solutions. This solution was drawn into the cell and its contents were shaken to effect complete mixing. The solution was transferred to a 50 ml volumetric flask. Ammonium molybdate (10 ml of 10% solution) and phosphoric acid (2 ml of 85% solution) were added with stirring. The flask was filled to the mark with double distilled water, and after one hour, the optical density was measured at 670 mμ.

In addition, some determinations have been made using the "methylene blue" technique.[74] In this method, p-aminodimethylaniline

sulfate (PADA) is oxidized to methylene blue in the presence of hydrogen sulfide under carefully controlled conditions. The tips of the sealed vessels containing irradiated solutions were broken under the surface of a solution of 5% zinc acetate and 1% sodium acetate (5 mls of each). The mixture was transferred to a 50 ml volumetric flask, and 5 ml of a freshly prepared dilute PADA solution was added (5 ml of 0.68 M PADA stock solution diluted to 250 ml with 1:1 sulfuric acid). The solution was shaken until it cleared. Immediately, two drops of 0.02 M ferric chloride in 1.2 M hydrochloric acid were added. After 10 minutes the mixture was diluted to the mark, and the optical density at 670 mμ was measured after one hour.

The yields of hydrogen sulfide from the mercaptan solutions were not determined quantitatively, but the production of hydrogen sulfide was confirmed by the methylene blue method. Methyl mercaptan also forms a coloured compound, which interferes with the determination of hydrogen sulfide. The molybdate method was tried with mercaptan samples, but again, a substantial blank was encountered, resulting in unsatisfactory reproducibility of results. Further work would be required to establish an analytical technique for the determination of hydrogen sulfide in methyl mercaptan solutions.

Neutral and alkaline sulfide solutions are susceptible to air oxidation, and during the course of this investigation a great deal of trouble was experienced in the preparation of calibration curves of optical density versus concentration of sulfide. Sulfide solutions were usually prepared by dissolving sodium sulfide in double distilled

water which had been purged with purified nitrogen. Immediately before use they were standardized with iodine either by direct titration or by back-titration with thiosulfate. The two methods gave the same result. Even with these precautions it was often difficult to get reproducible results due to the instability of the sulfide solutions. Two other sources of sulfide ion were tried, cadmium sulfide and ammonium sulfide. Neither of these gave better (or more reproducible) results than did the sodium sulfide.

An alternative procedure for obtaining the standard curves has therefore been developed in this laboratory. The apparatus shown in Figure 6 consists of two 2-way stopcocks, A and B, joined to form a standard volume, C, between their barrels. This volume was calibrated with mercury. The apparatus was assembled as shown with about 10 ml of water in bulb D. This water and all connecting tubing were purged with nitrogen and tube F was flushed with water. After a second purging with nitrogen, C was flushed for several minutes with gaseous hydrogen sulfide, the gas being exhausted through E. Following this A and B were closed in that order. B was then opened to the water reservoir D and A was opened to allow the hydrogen sulfide (gas) in C to be swept into the thiol solution in the volumetric flask. Tube F was then rinsed and the colorimetric reagents were added to the flask.

A lecture bottle containing hydrogen sulfide of better than 99% purity was used as the source of this gas. The exact quantity of hydrogen sulfide was calculated from the volume of the capillary, atmospheric pressure, and temperature. While this method provided for

Figure 6

Figure 6

Gas Measuring Apparatus

A -- 2-way stopcock

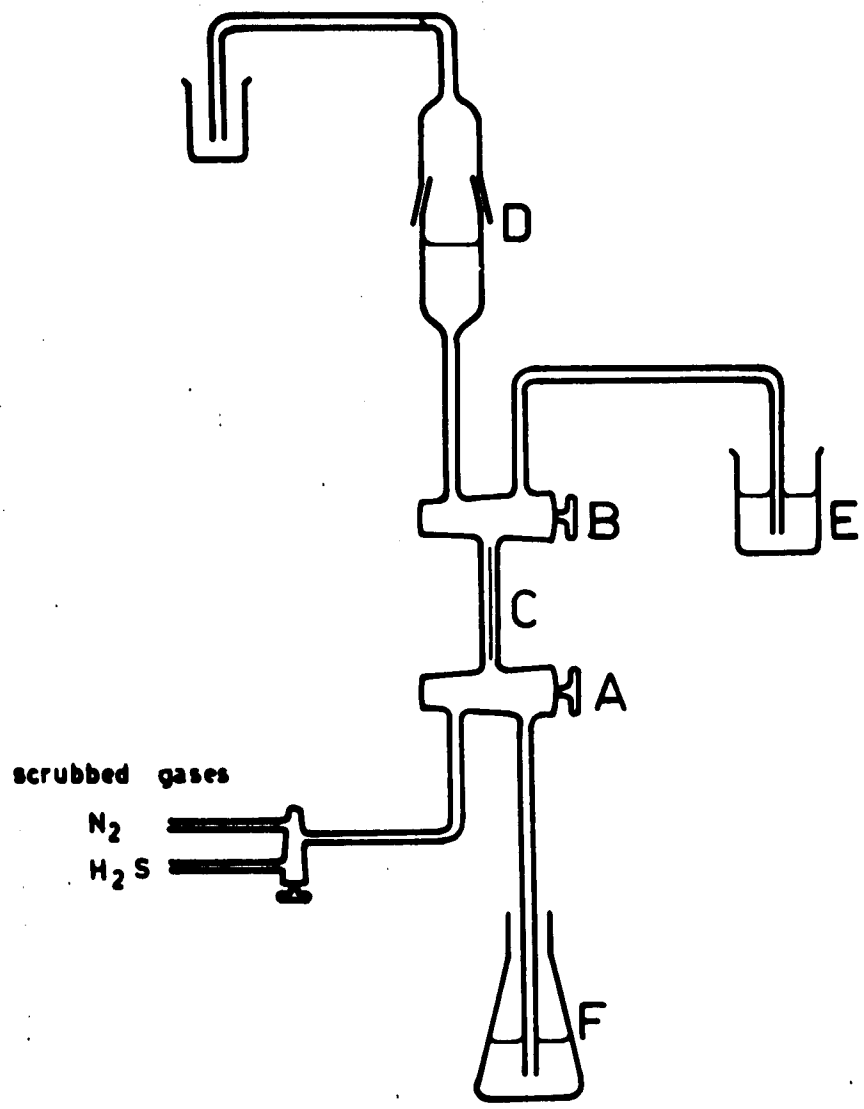
B -- 2-way stopcock

C -- Standard volume

D -- Water reservoir

E -- Gas exhaust

F -- Tube


D
B
E
C
A
scrubbed gases
N_2
H_2S
F

more reproducibility in the sulfide ion content of the standard solutions, it had a serious drawback in that a different apparatus had to be constructed for every volume of hydrogen sulfide to be measured.

It was found that the above method of measuring sulfide content gave calibration curves which were consistent within experimental error with those obtained by using sodium sulfide only if the sodium sulfide solutions had been used immediately after preparation. This applied also to solutions of cadmium sulfide and ammonium sulfide. If the sulfide solutions had been allowed to stand for 6 hours or longer the slopes of the standard curves of optical density versus sulfide concentration varied widely. It appears that upon standing the sulfide solution decomposes, giving rise to products which do not react with I_3^- but which interfere with the development of the colour in the hydrogen sulfide analyses. The effect of acid on the standard curves was investigated and found to be negligible.

Previous investigation of the absorption of cysteine and cystine at 254 mμ[75] showed that the molar extinction coefficient for cystine ranges from 3.5 to 10^3, depending on pH. In the present study the value of ϵ_{max} for cystine and the effect of pH on it were studied. Since the rate of oxidation of cysteine to cystine is slow at low pH, acid solutions of cysteine containing known amounts of cystine were prepared and the optical density measured at 248 mμ. The presence of cysteine was required to reproduce the conditions existing in the irradiation cell. Cysteine solutions containing no cystine were used as reference. At this wavelength and pH = 0, the molar extinction

coefficient was found to be 300. This is to be compared with a value of 345 at 248 mμ in acid solutions reported by Greenstein.[76]

Cystine does not dissolve readily in neutral solutions. Therefore it was necessary to dissolve known amounts of cystine in an acidified cysteine solution. Aliquots of this solution were then neutralized with potassium hydroxide, in the presence of ammonium acetate buffer. All solutions were thoroughly purged with purified nitrogen before this procedure was begun. The pH was measured after the optical densities were taken. Since the exact volumes and concentrations were known, the ε_{max} could be determined. It was found to be 324 in the pH range 5 to 6.

In order to measure quantitatively the yield of cystine produced in irradiated cysteine solutions, the optical density of the irradiated system was measured at 248 mμ using a Beckman DU Spectrophotometer. In neutral solutions, oxygen had to be rigorously removed to eliminate oxidation, so the special cell shown in Figure 4 was used. Aqueous solutions of cysteine were purged for 30 - 45 minutes with purified nitrogen, and then 5 ml samples degassed by the usual procedure of freezing, pumping and thawing. After the vessel had been sealed off under vacuum, the sample was melted and poured into the quartz spectrophotometer cell. The optical density of the unirradiated system at 248 mμ was then determined. The solution was tipped back into the irradiation cell for exposure to the Co^{60} γ-rays. Measurements of the optical density at various doses were carried out in this manner.

The yield of alanine from irradiated cysteine solutions

was determined in collaboration with Dr. M. Lal,[77] using the Amino Acid Analyzer which was described earlier in the Apparatus section.

Some methane determinations were made by means of mass spectrometry.

3. RESULTS

The major products of the radiolysis of aqueous solutions of cysteine are cystine, hydrogen, hydrogen sulfide and alanine. These have been measured over a range of doses, for several different initial concentrations of cysteine, and at varying values of pH. The effects of these three variables will be presented and discussed in that order.

No hydrogen could be detected from unirradiated samples of cysteine solutions analyzed immediately after preparation and after having stood for several hours at room temperature. Likewise no alanine was produced from the unirradiated systems. Unirradiated solutions of cysteine were used as reference samples in the spectrophotometric determination of hydrogen sulfide and cystine.

3.1 Dose Dependence

Figure 7 shows the dose dependence of the hydrogen yields from 10^{-4} M and 10^{-3} M cysteine solutions. The specific yields for 10^{-3} M solutions are somewhat less dose dependent than those for the more dilute solutions, the independent region extending from (0.7 to 2.5) x 10^{18} eV/ml. The specific yields from more concentrated solutions (10^{-2} M and 10^{-1} M) vary even less with dose, being independent from (0.9 to 4) x 10^{18} eV/ml at pH 0 (Figure 8).

The variation of hydrogen sulfide yields with dose for 10^{-3} M and 10^{-2} M cysteine solutions are indicated in Figure 9. As with the hydrogen yields, the dose dependency of the specific yields from the 10^{-2} M solutions is much less pronounced than those from 10^{-3} M solutions.

Figure 7

Figure 7

Dose Dependence of Hydrogen Yields for Irradiated Cysteine Solutions

o -- 10^{-4} M cysteine solution, pH = 3.5

● -- 10^{-3} M cysteine solution, pH = 3.3

● -- 10^{-3} M cysteine solution, pH = 0 - 1

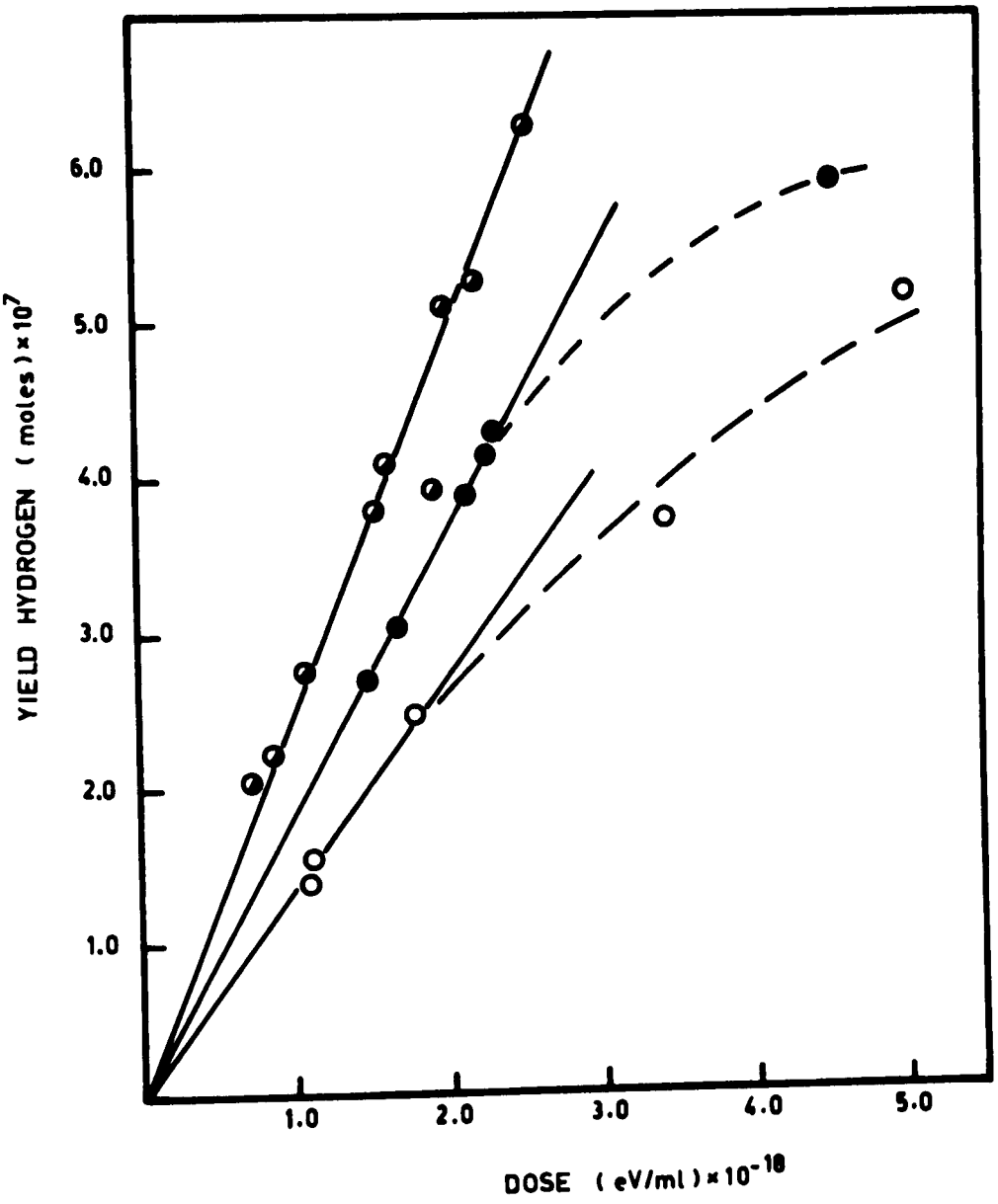

YIELD HYDROGEN (moles) × 10^7
6.0
5.0
4.0
3.0
2.0
1.0
1.0
2.0
3.0
4.0
5.0
DOSE (eV/ml) × 10^-18

Figure 8

Figure 8

Dose Dependence of Hydrogen Yields for Irradiated Cysteine Solutions

o -- 10^{-2} M cysteine solution, pH = 0

● -- 10^{-1} M cysteine solution, pH = 5

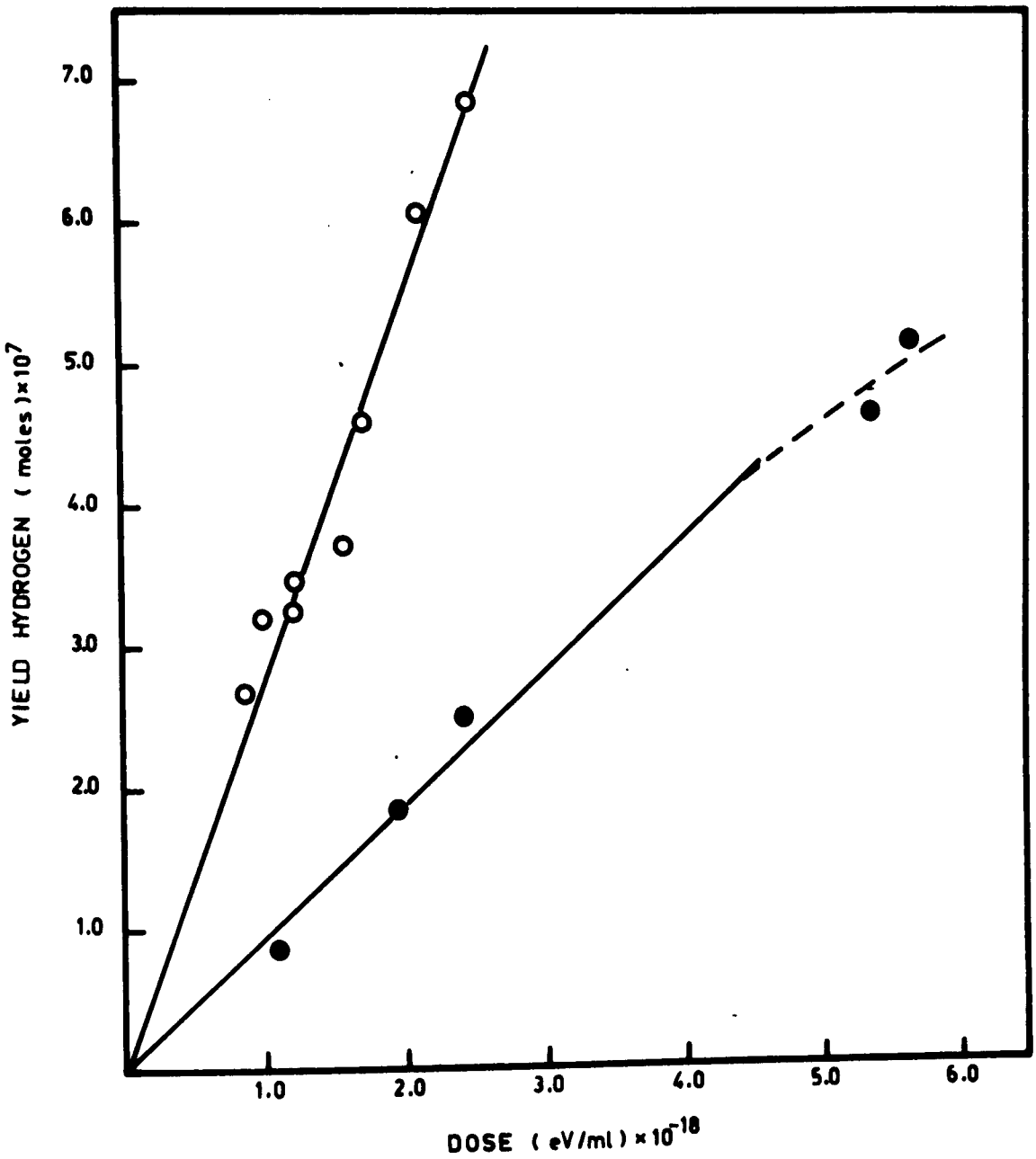

YIELD HYDROGEN (moles)×10^7
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1.0
2.0
3.0
4.0
5.0
6.0
DOSE (eV/ml) × 10^-18

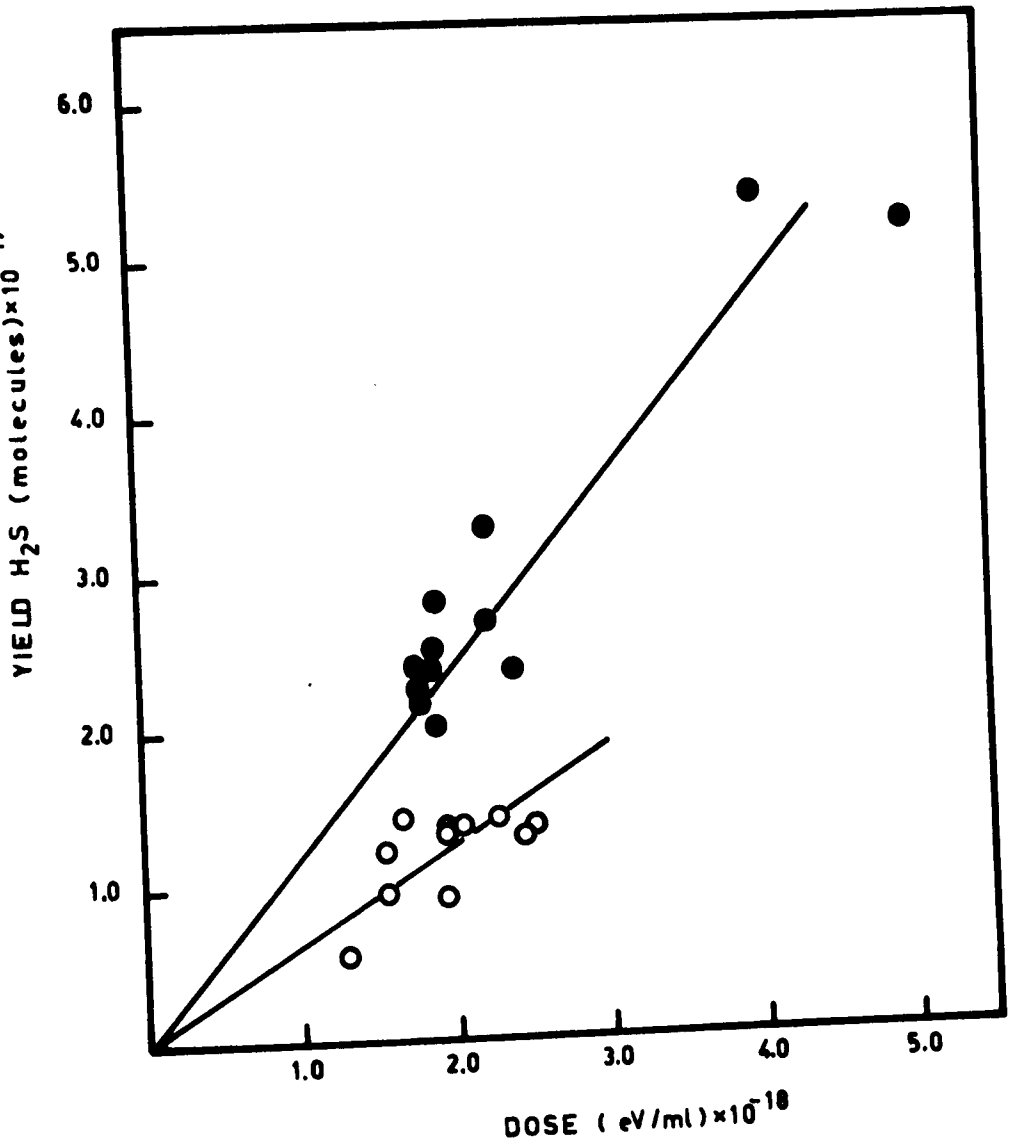
Figure 9

Figure 9

Hydrogen Sulfide Yields as a Function of Dose for Irradiated Cysteine Solutions

o -- 10^{-3} M cysteine solution, pH = 5.5

● -- 10^{-2} M cysteine solution, pH = 5.5


6.0
5.0
4.0
3.0
2.0
1.0
YIELD H_2S (molecules)$\times 10^{-17}$
1.0
2.0
3.0
4.0
5.0
DOSE (eV/ml)$\times 10^{-18}$

Alanine yields as a function of dose are shown in Figure 10 for 10^{-3} and 10^{-2} M cysteine solutions at neutral pH, and Figure 11 for acid solution. Again it is noted that there is far less dependence upon dose in the more concentrated solutions over the range 0 - 10^{19} eV/ml. The variation of hydrogen sulfide yields is shown on the same graph. The significance of this will be discussed later.

Yields of cystine were determined spectrophotometrically. A plot of optical density versus total dose (Figure 12) shows that the specific yields are dose dependent in the dilute solutions, and nearly independent in the more concentrated solutions.

3.2 Concentration Dependence of Hydrogen Sulfide Yields

The hydrogen sulfide yields were studied as a function of concentration in somewhat greater detail than the yields of other major products. The results of this study for cysteine concentrations from 10^{-3} M to 10^{-2} M at pH 5.5 - 6.8 and at a constant dose of $\sim 2 \times 10^{18}$ eV/ml are shown in Figure 13. Each point on the graph represents the mean of 4 - 12 separate determinations. Where possible standard deviations were calculated and are indicated on the graph by the vertical lines through the points. The G-value increases sharply from the initial value of ~ 1 at 10^{-3} M cysteine to ~ 2.5 at 3×10^{-3} M, then maintains an apparent plateau at 2.6 up to cysteine concentration of 10^{-2} M.

3.3 Variation of Product Yields with pH

For both 10^{-3} and 10^{-2} M cysteine solutions a study of the variation of $G(H_2)$ and $G(H_2S)$ over the pH region 0 - 7 was made. The

Figure 10

Figure 10

Dose Dependence of Hydrogen Sulfide and Alanine Yields for Irradiated Cysteine Solutions at pH = 4.5 - 6

o -- Alanine, [cysteine] = 10^{-2}M

● -- Hydrogen sulfide, [cysteine] = 10^{-2}M

● -- Alanine, [cysteine] = 10^{-3}M

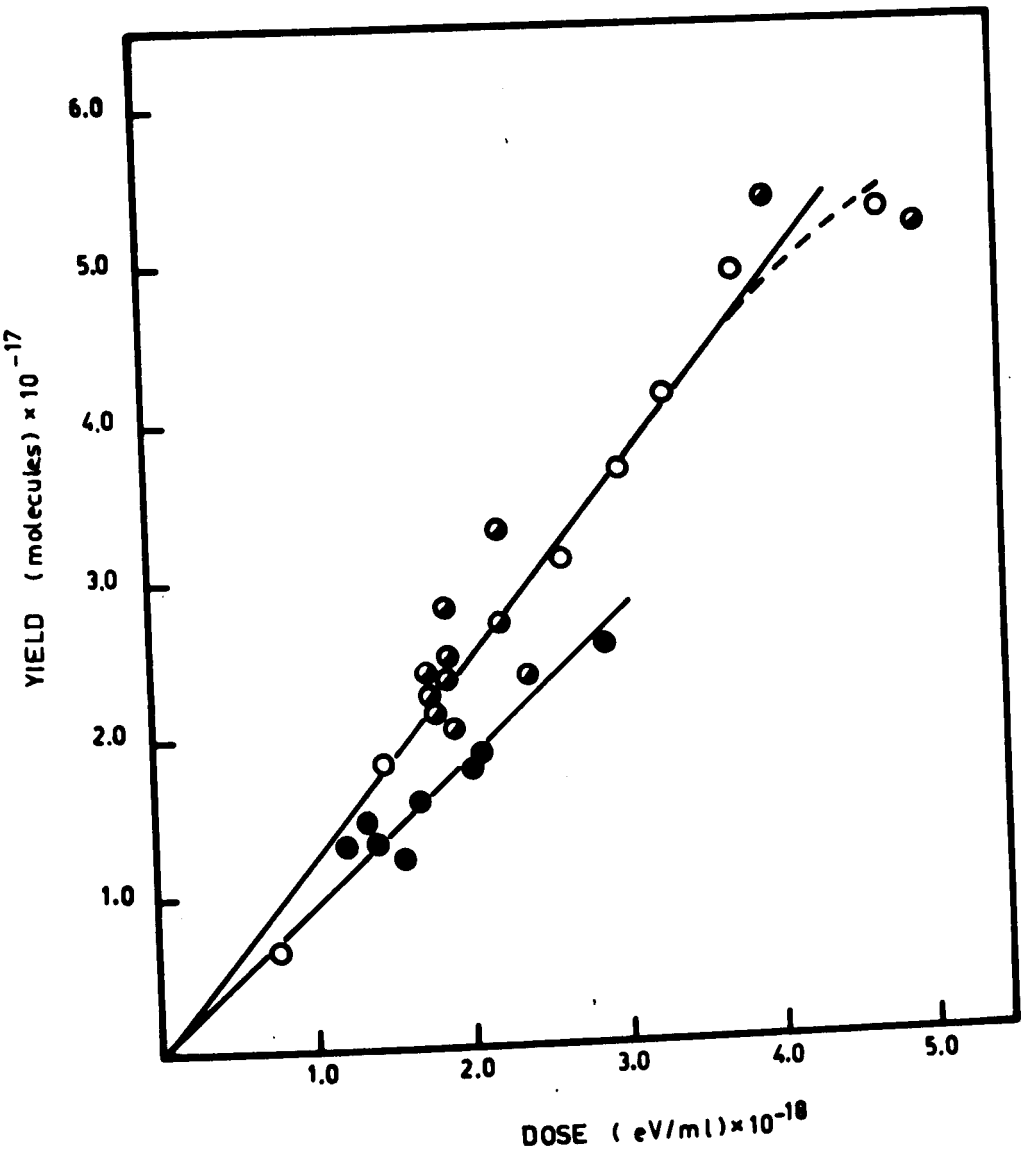

6.0
5.0
4.0
3.0
2.0
1.0
YIELD (molecules) × 10⁻¹⁷
1.0
2.0
3.0
4.0
5.0
DOSE (eV/ml) × 10⁻¹⁸

Figure 11

Figure 11

Dose Dependence of Alanine Yields for Irradiated Cysteine Solutions at pH = 0 - 1

o -- 10^{-3} M cysteine solution, pH = 0

● -- 10^{-2} M cysteine solution, pH = 0 - 1

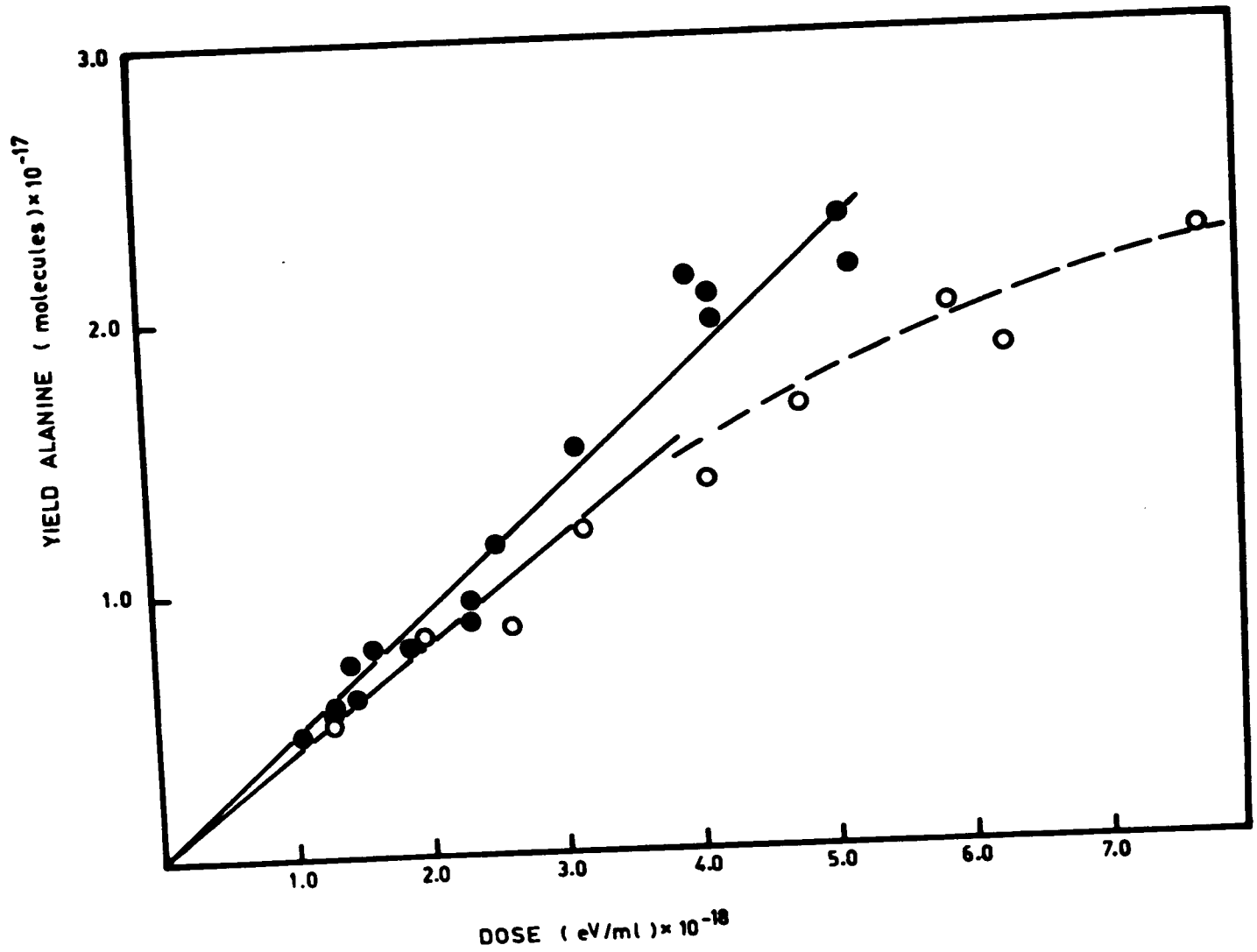

3.0
2.0
1.0
YIELD ALANINE (molecules) × 10⁻¹⁷
1.0
2.0
3.0
4.0
5.0
6.0
7.0
DOSE (eV/ml) × 10⁻¹⁸

Figure 12

Figure 12

Plot of Δ Optical Density versus Dose for the Determination of Cystine from Irradiated Cysteine Solutions

o -- 10^{-3} M cysteine solution, pH = 5.5

● -- 10^{-2} M cysteine solution, pH = 4

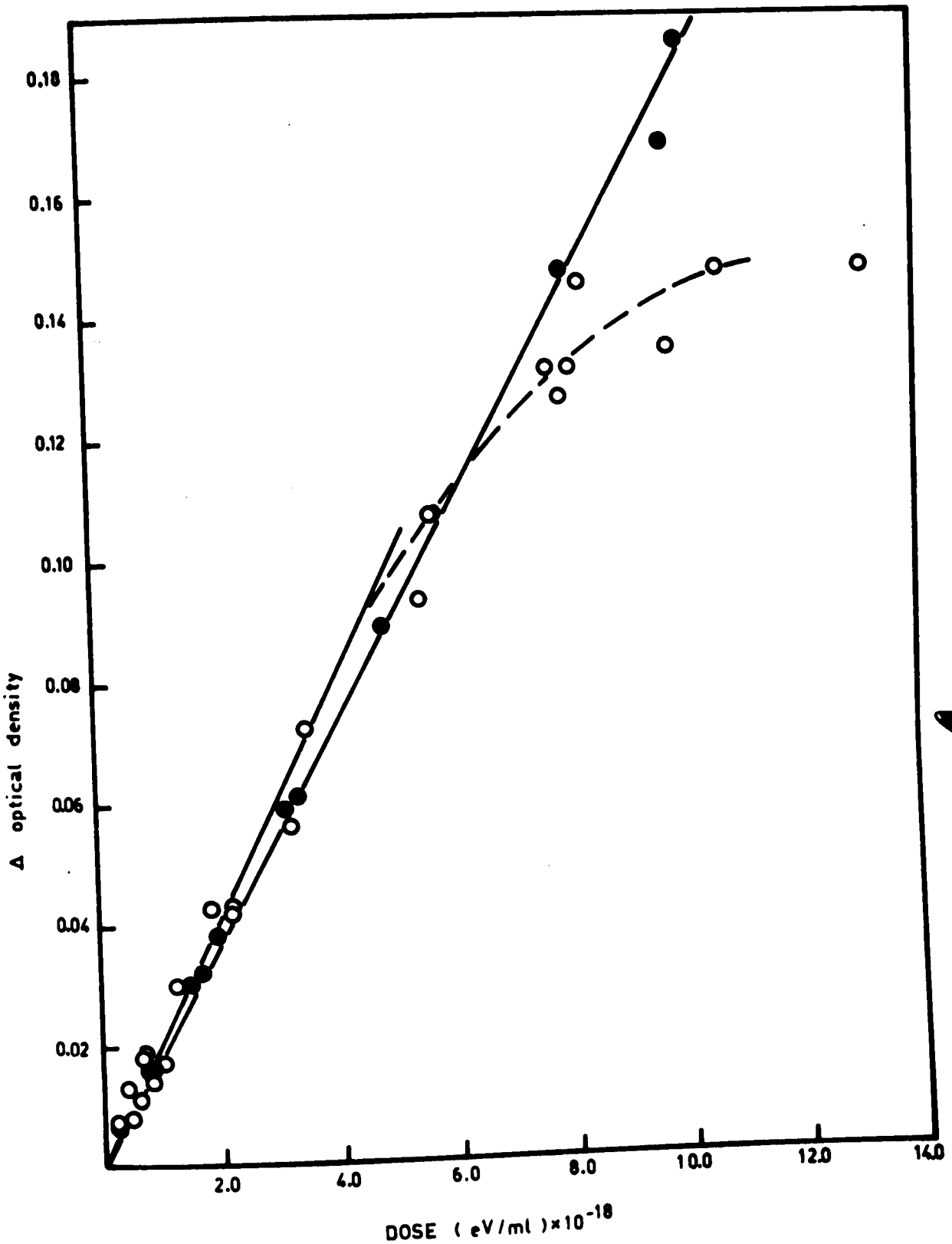
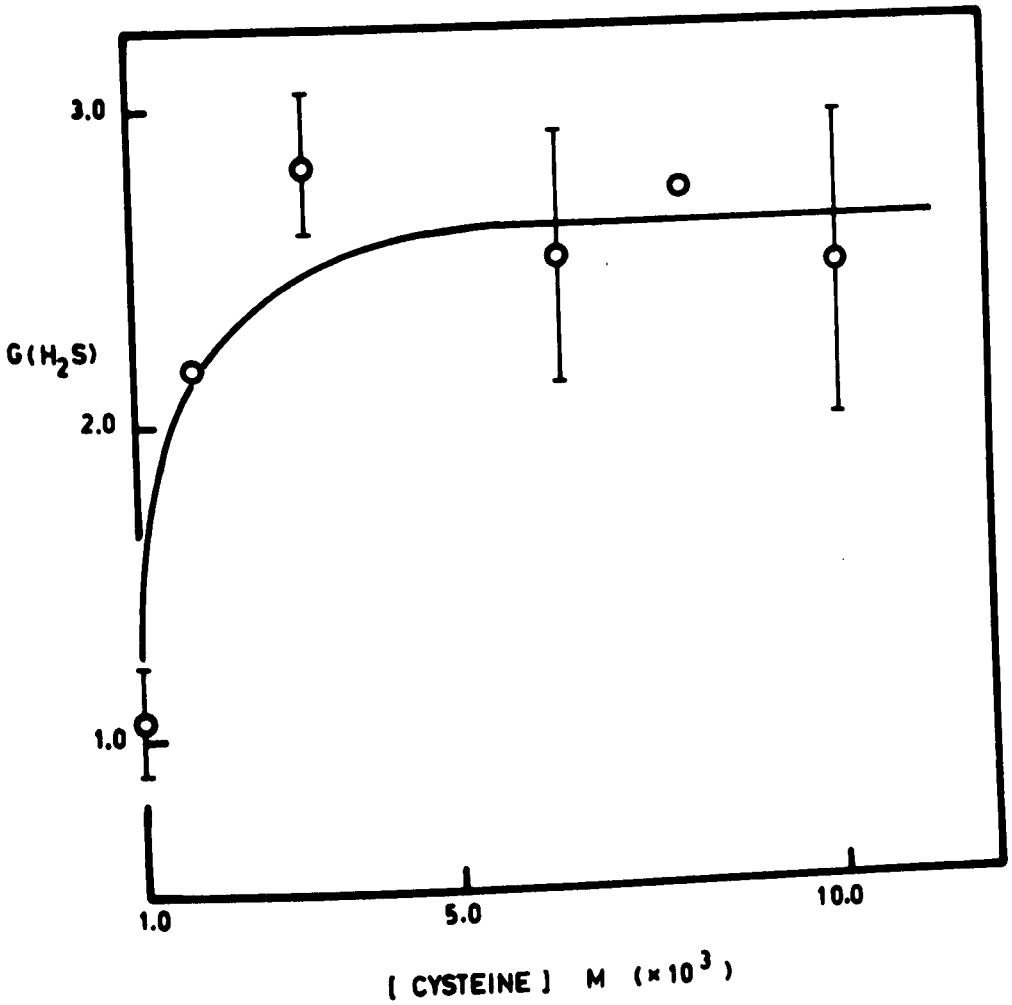

0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
Δ optical density
2.0
4.0
6.0
8.0
10.0
12.0
14.0
DOSE (eV/ml)×10⁻¹⁸

Figure 13

Figure 13

$G(H_2S)$ as a Function of Cysteine Concentration at Neutral pH


3.0
2.0
1.0
G(H2S)
1.0
5.0
10.0
[CYSTEINE] M (×10 3)

results are shown in Figures 14 and 15, in which perchloric acid was used to vary the pH. Each point represents a mean of 2 - 12 individual determinations. Standard deviations were calculated where possible, and are indicated by the vertical lines through the points. The G-value for hydrogen decreases as the pH is varied from 0 - 6, the value at which it sharply decreases occurs at higher values of pH as the solute concentration decreases. The G-value for hydrogen sulfide increases as the pH is varied from 0 - 6. The variation with concentration at this pH range follows a pattern similar to that found with the hydrogen yields. For an initial concentration of 10^{-2} M cysteine, G(Alanine) was studied as a function of pH. The results are also shown in Figure 15. As was found with the hydrogen sulfide yields, G(Alanine) increases with increasing pH. Within experimental error, the sharp rise occurs at the same point as that for $G(H_2S)$, which would be expected. This agreement will be discussed later. G(Alanine) for 10^{-3} M cysteine solutions was determined under acid and neutral conditions only (Table II). The trend of increased alanine yield with increased pH observed with 10^{-2} M solutions appears to hold for more dilute solutions, but the increase is not as great as that found for 10^{-2} M solutions.

Cystine has been measured for both 10^{-3} and 10^{-2} M cysteine solutions under acid and neutral conditions. The results are given in Table II, and each value represents the mean of 2 - 5 determinations. It should be noted that for 10^{-2} M solutions, G(Cystine) decreases with increasing pH, falling from 4.2 to 3.4 as the pH changes from 0 - 6, whereas for 10^{-3} M solutions, the G-value increases from 3.1 to 3.8 over the

Figure 14

Figure 14

Variation of $G(H_2)$ and $G(H_2S)$ with pH for 10^{-3} M Cysteine Solutions

● -- Hydrogen

o -- Hydrogen sulfide

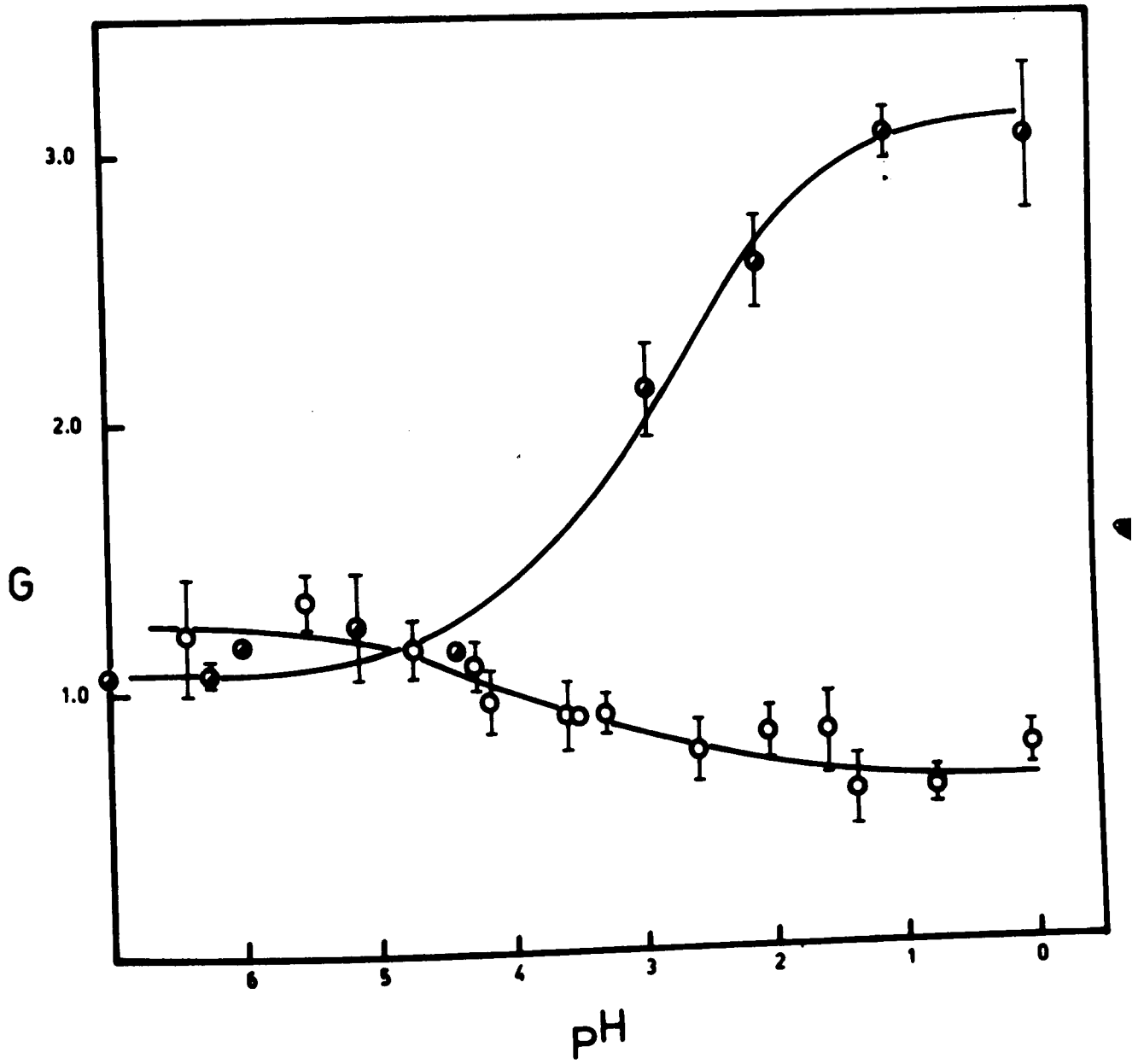

3.0
2.0
1.0
G
6
5
4
3
2
1
0
pH

Figure 15

Figure 15

pH Dependence of $G(H_2)$, $G(H_2S)$, and G(Alanine) for 10^{-2} M Cysteine Solutions

- ● -- Hydrogen
- o -- Hydrogen sulfide
- ● -- Alanine

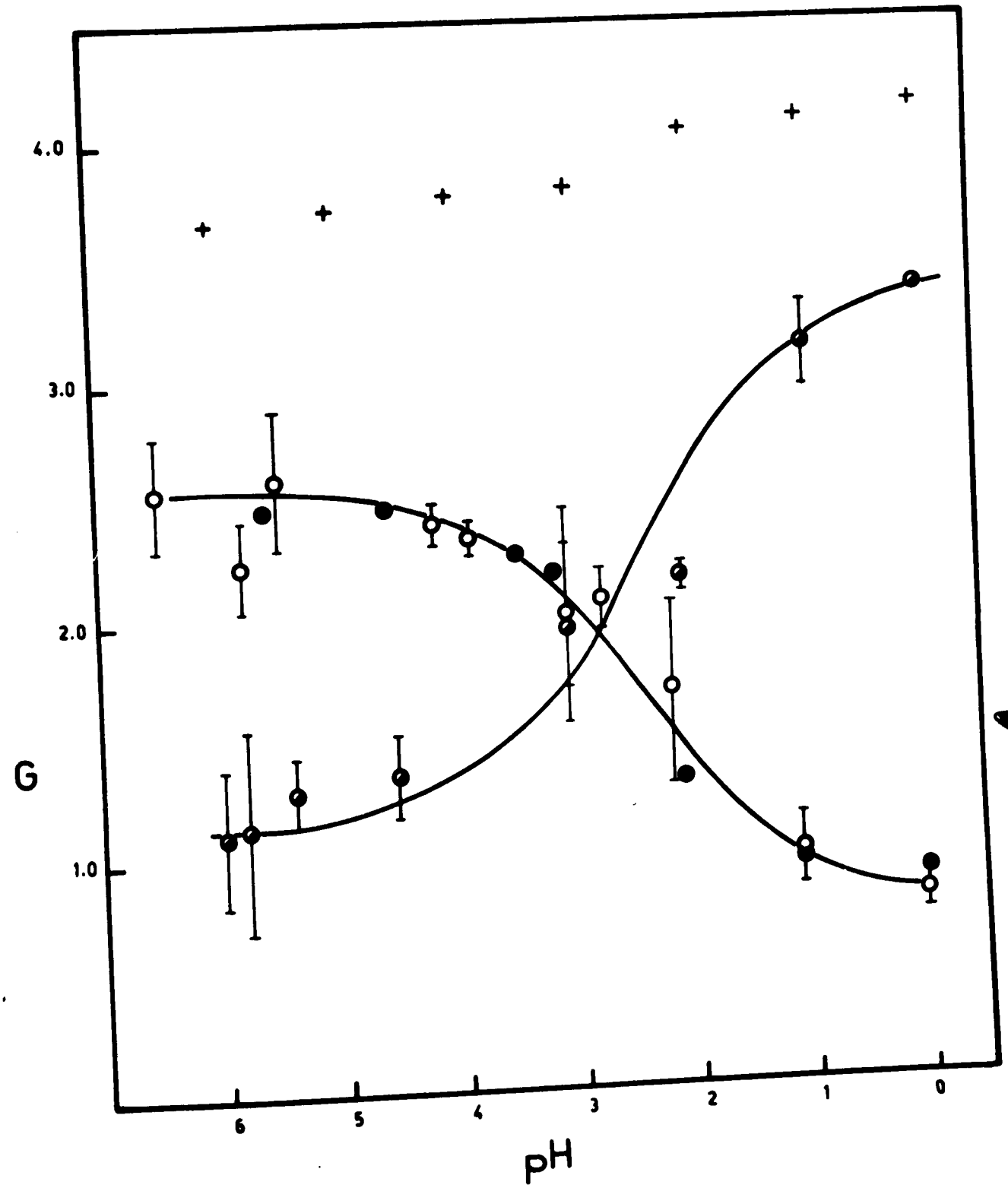

4.0
3.0
2.0
1.0
G
6
5
4
3
2
1
0
pH

TABLE II

Yields of Alanine and Cystine

Cysteine	pH	G(Alanine)	G(Cystine)
10^{-2} M	0	0.86	4.2
	5	2.50	3.4
10^{-3} M	0	0.85	3.1
	5	2.18	3.8

same pH range.

3.4 Other Studies

Since hydrogen sulfide formation was believed to be due to a reaction of solvated electrons with cysteine (see Introduction and Discussion sections) known electron scavengers, with very high rate constants for reaction with e^-_{aq}, were introduced into the system. These scavengers should compete with the cysteine for the solvated electron and should reduce the yields of hydrogen sulfide. The results of competition with acetone are shown in Figure 16, and those from a similar competition with nitrate ion in Figure 17. In both cases the hydrogen sulfide yields were reduced to low levels when the additive was present in high concentration. With added acetone the value of $G(H_2S)$ was about 0.70 at a [scavenger]/[cysteine] ratio of 10. Added nitrate reduced the hydrogen sulfide yield to a G-value of $\sim$0.38 at a [scavenger]/[cysteine] ratio of 10.

Figure 16

Figure 16

Variation of $G(H_2S)$ with [Acetone]/[Cysteine]

for 10^{-2} M Cysteine Solutions

at pH = 5

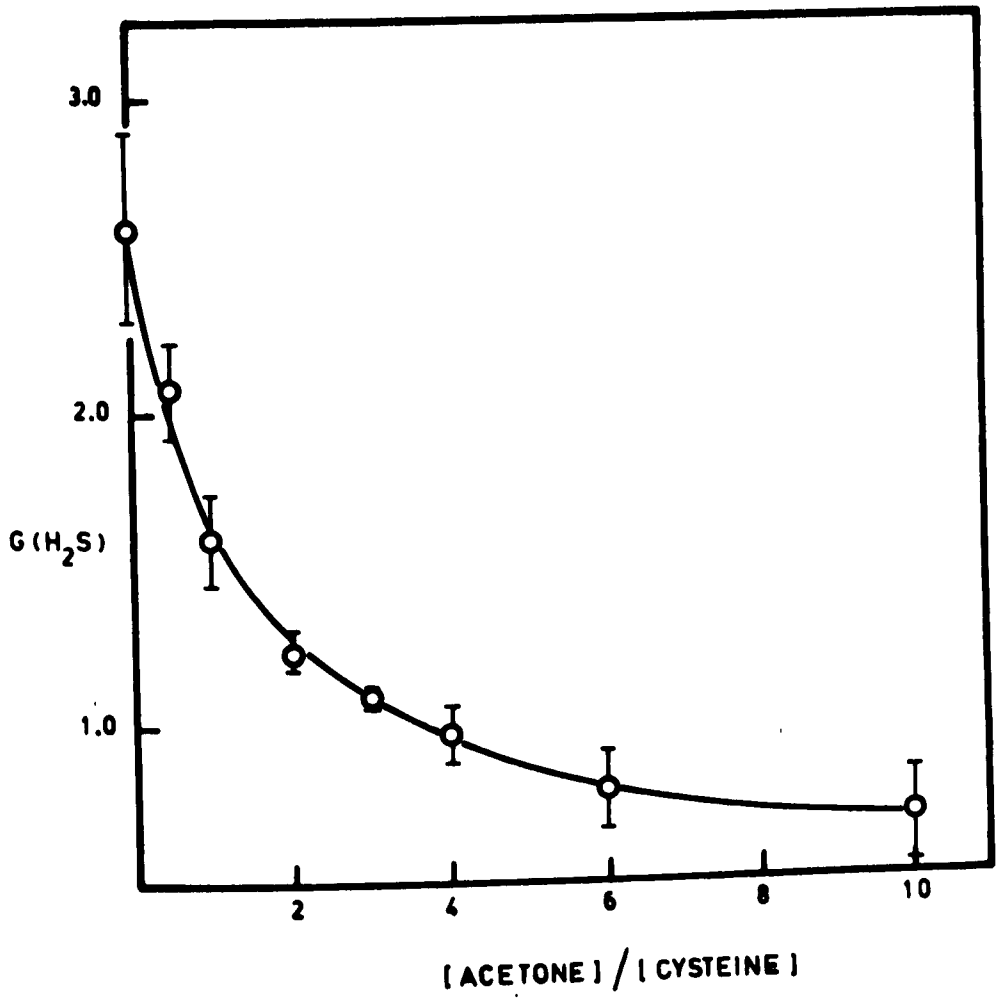

3.0
2.0
1.0
G(H2S)
2
4
6
8
10
[ACETONE] / [CYSTEINE]

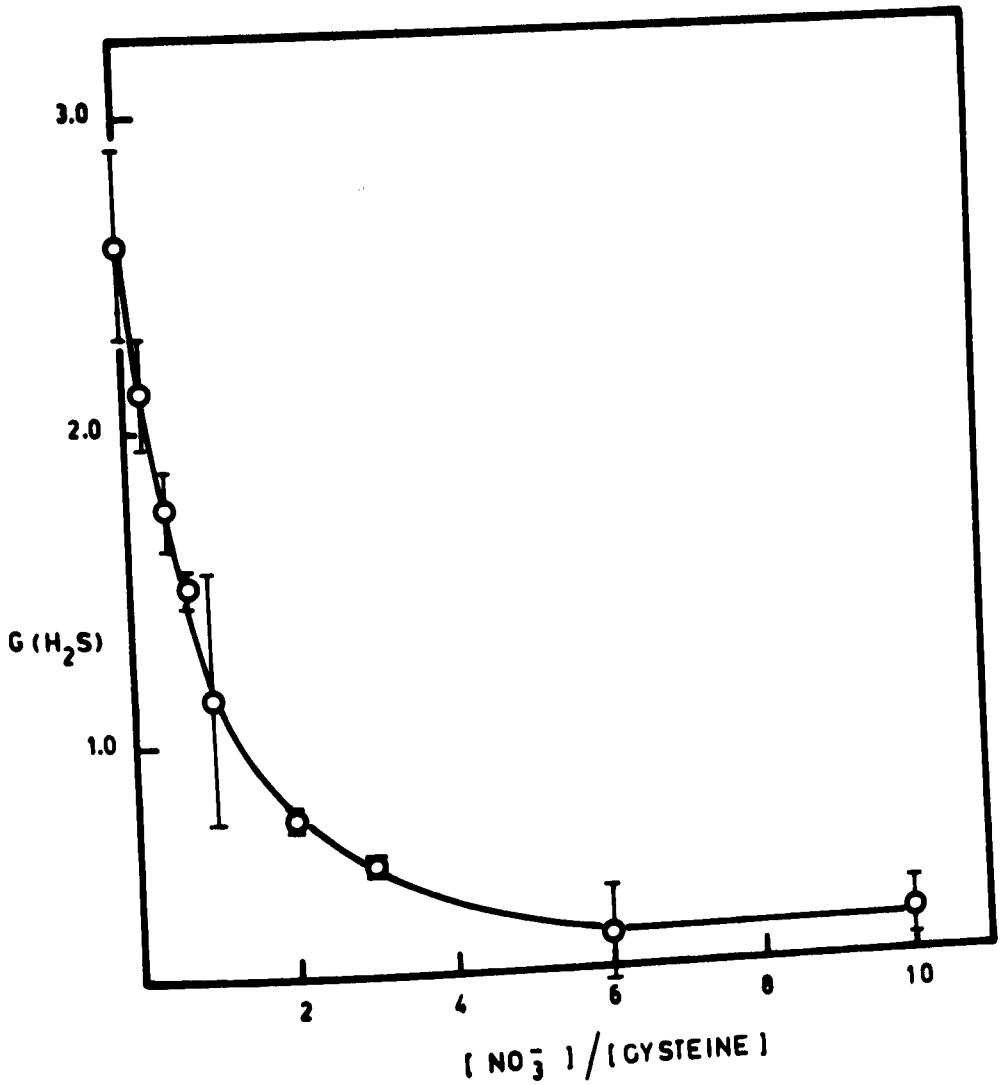
Figure 17

Figure 17

Variation of $G(H_2S)$ with $[NO_3^-]/[Cysteine]$

for 10^{-2} M Cysteine Solutions

at pH = 5


3.0
2.0
1.0
G (H2S)
2
4
6
8
10
[NO3-] / [CYSTEINE]

4. DISCUSSION

From the data obtained in the present study and that available from previous studies by other workers,[63,64,65,67,68] it is clear that the major products from exposure of cysteine in oxygen-free solutions to low doses of ionizing radiation are cystine, hydrogen, hydrogen sulfide, and alanine.

At higher doses secondary reactions may occur which could, conceivably, lead to small amounts of products which have not been detected. One such product would be ammonia. Other workers[65,67] have noted that deamination, which is important in the radiolysis of other amino acids, does not occur appreciably with cysteine. However trace amounts could be formed either from direct deamination of cysteine, or from radiolysis of the primary products alanine and cystine. Both the latter reactions are known,[65,78] and it has been established[55] that cystine is more susceptible to radiation deamination than is cysteine. Deamination of cysteine occurred under the radiation conditions obtaining in this study as evidenced by the distinct odour of ammonia in equilibrium with very dilute, neutral solutions. The deamination reaction was not studied quantitatively. Decarboxylation is another possible secondary reaction. By using a barium hydroxide trap, Markakis *et al*[65] observed that a large amount of carbon dioxide was formed at high doses. Therefore, it would appear that decarboxylation is also important in the overall pattern of cysteine radiolysis at large doses. However, this reaction has not been studied quantitatively.

Markakis *et al*[65] also noted the presence of a "cooked cabbage-like odour in solutions which had been subjected to large

doses, but this gas was not identified. In the present study a "cooked rhubarb-like" odour was noted for 10^{-2} M cysteine in acid solutions and at low doses, but again this has not been identified. The odour of these unidentified gases suggests the presence of some minor sulfur-containing product(s) in addition to hydrogen sulfide.

The results of this study will be discussed under the following broad headings:

(1) Dose dependence

(2) Effect of concentration

(3) Total radical yield

(4) Effect of pH

(5) Competition kinetics

(6) Total decomposition of cysteine

(7) Comparison with other related thiols

(8) Implications for radiation biology

4.1 Dose Dependence

In the present study it has been shown (Figures 7 to 12) that at initial cysteine concentrations of 10^{-3} M and lower, the specific yields of all the products are very dose dependent. It is well known that G-values are often dose dependent because the products which accumulate may compete with the original solute for reactive intermediates. When the products are as reactive as are hydrogen sulfide and cystine, the chances of secondary reactions are much enhanced. As a result one tries to measure yields at as low a dose as possible. In such cases extremely small quantities of product are formed, and unless the analytical techniques are sufficiently sensitive, large errors in

the analysis occur. For example, in the determination of hydrogen sulfide at doses less than 0.8×10^{18} eV/ml, it was necessary to make measurements not far from the limit of detection. The scatter in the results shows this.

In Figure 9, there is some suggestion of a dose dependence, although the range of doses used is too short for this to be clear. El Samahy[70] has shown a dose dependence of hydrogen sulfide for 10^{-3} M cysteine solutions under γ-irradiation. Alanine yields are clearly dose dependent (see Figures 10 and 11), and it is not unreasonable to suspect that the hydrogen sulfide results in 10^{-3} M solutions are also dose dependent.

Studies on the radiolysis of both hydrogen sulfide and cystine have been carried out in detail.[65,72] In addition the effect of atomic hydrogen on aqueous cystine has been investigated.[79] Both compounds have been shown to react rapidly with hydrogen atoms and solvated electrons, so that competition of these products as they accumulate with cysteine for the reactive intermediates is not unexpected. This problem is increased as the solute concentration is reduced. Assuming $G(\text{Cystine}) \sim 3$, it can be shown that starting with 10^{-4} M cysteine solutions, the concentration of cystine would approach that of cysteine at a dose of $\sim 10^{18}$ eV/ml.

Mechanistic conclusions can usually be drawn only from dose independent G-values or true initial yields. Hence, discussion of the data obtained in this investigation will tend to emphasize the more concentrated solutions where the range for dose independence is greater. In all cases the radiolytic yields are reported only for initial slope

data, and are given as G-values - the number of molecules formed or destroyed per 100 eV of energy absorbed in the system.

4.2 Effect of Concentration

The variation of hydrogen sulfide yields with cysteine concentration at neutral pH was studied briefly. The results are shown in Figure 13, for cysteine concentrations ranging from 10^{-3} to 10^{-2} M. If the low yields observed in 10^{-3} M solutions are attributed to interaction of hydrogen sulfide with solvated electrons and hydrogen atoms, then as the cysteine concentration is increased the yield of hydrogen sulfide should rise up to the value normally accepted as corresponding to that of the radical giving rise to hydrogen sulfide and then remain relatively independent of solute concentration. The concentration dependence of $G(H_2S)$ (see Figure 13) agrees with this. The plateau value of $\sim$2.6 molecules/100 eV corresponds well with the accepted yield of the solvated electron in neutral irradiated water (2.7 ± 0.2). This agreement tends to support reaction (54) as a source of hydrogen sulfide.

During the course of this work, Trumbore *et al*[69] published some of the results of their investigation of γ-irradiated aqueous cysteine solutions. It was noted that the disappearance of the thiol group, expressed as G(-RSH), was quite sensitive to initial concentration of cysteine. The authors suggested that a relatively simple mechanism at lower initial cysteine concentration (2×10^{-4} M) may change to a more complex partial chain mechanism at higher initial

solute concentration (4×10^{-3} M). It is clear from the concentration study of the hydrogen sulfide yields carried out by the author that a chain mechanism, if it does occur, does not involve formation of hydrogen sulfide. If it did, then a levelling off of the G-value with increasing cysteine concentration would not have been expected. Furthermore, if there are chain reactions, then the reasonable agreement found between product yields and known radical yields could not be explained. This latter point will be elaborated further in the sections: Total radical yield and Effect of pH.

It is found that G(Alanine) and $G(H_2)$ for 10^{-3} M cysteine solutions approach those for 10^{-2} M cysteine solutions as the dose approaches zero at the same pH. If the "low" values of hydrogen sulfide found in 10^{-3} M solutions are actually due to reactions with the reactive intermediates formed in water, then it is apparent that hydrogen sulfide yields must be measured at lower doses. In other words, the concentration dependence of $G(H_2S)$ shown in Figure 13 is probably due to the use of data which are not true initial yields. However, due to the lower sensitivity of the analytical techniques, it is impossible to obtain a meaningful yield of hydrogen sulfide at doses lower than 0.8×10^{18} eV/ml for 10^{-3} M solutions.

If the true initial yield of hydrogen sulfide for 10^{-3} M solutions is in fact close to that for 10^{-2} M solutions, as is found with G(Alanine) and $G(H_2)$, then it would seem that the radiolytic mechanism for the formation of these three products is not concentration dependent.

4.3 Total Radical Yield

It has been shown in earlier studies[64,66] that the yield of hydrogen peroxide which accumulates in irradiated air-free acid solutions of cysteine is consistent with the accepted molecular yield, $G^{M}_{H_2O_2}$. The fact that cysteine and hydrogen peroxide are stable together in acid solution was recently confirmed by El Samahy.[70] Thus it is reasonable to conclude that the molecular hydrogen peroxide formed in acid solutions does not react with cysteine or affect its decomposition provided its concentration never rises to the point where it begins to compete for radical intermediates. Obviously similar considerations should apply to the molecular hydrogen.

It has been assumed[63,64,65,66] that hydroxyl radicals will abstract hydrogen atoms from the thiol group:

$$OH + RSH \longrightarrow H_2O + RS \qquad (33)$$

It has recently been shown[69] that the reaction between hydroxyl radicals and cysteine is rapid. A nearly diffusion-controlled reaction rate constant of $\sim 3 \times 10^9\ \bar{M}^1\ sec^{-1}$ was found by means of competition kinetic studies.

As discussed in an earlier section, there is evidence that the reactions of the reducing radicals, e^-_{aq} and H, with cysteine are highly complex. Littman *et al*[68] proposed the following reactions to explain the results of their investigation of the reactions between hydrogen atoms and cysteine:

$$2\ RS \longrightarrow RSSR \qquad (52)$$

$$H + RSH \longrightarrow H_2 + RS \quad (37)$$

$$H + RSH \longrightarrow H_2S + R \quad (43)$$

Reaction (43) would be followed by (47)

$$R + RSH \longrightarrow RH + RS \quad (47)$$

It has been suggested[69,80] that attack of the solvated electron on cysteine produces hydrogen sulfide

$$e^-_{aq} + RSH \longrightarrow R + SH^- \quad (54)$$

Reaction (54) would also be followed by reaction (47). It may be seen from the reactions listed above that attack of each radical produced from irradiated water results in formation of one RS radical. If all the RS radicals combine as shown in reaction (52), then a study of the formation of cystine should give some idea of the total radical yield from radiolyzed water. From the proposed reactions it may be seen that in acid solutions

$$G_{RS} = G_{e^-_{aq}} + G_H + G_{OH}$$

$$= G_{red} + G_{OH}$$

G_X refers to the number of radicals, X, produced per 100 eV of energy absorbed, and G_{red} refers to the number of reducing radicals per 100 eV. Taking the characteristic radical yields from the literature,[11] G_{RS} may be shown to be 6.55. Therefore, G(Cystine) should be about 3.3. Markakis and Tappel[65] observed G = 4.4 in 0.1 M solution at pH 1.6. El Samahy[70] found in acid solution G = 1.2 and 4.0 for 8×10^{-4} M and 6×10^{-3} M

solutions, respectively. The present study yielded, at pH 0, G(Cystine) of 3.1 and 4.2 for 10^{-3} and 10^{-2} M solutions respectively.

El Samahy[70] showed from his studies of hydrogen peroxide and cysteine mixtures that in neutral solutions the following reaction occurs:

$$2\ RSH + H_2O_2 \longrightarrow RSSR + 2\ H_2O \qquad (51)$$

Thus, in neutral solutions, it may be seen that G(Cystine) is then equal to

$$\frac{G_{RS}}{2} + G^{M}_{H_2O_2}$$

$G^{M}_{H_2O_2}$ refers to the "molecular" hydrogen peroxide generated in irradiated water. G(Cystine) in neutral solutions should therefore be 3.5. El Samahy[70] observed a value of 2.8 for 9.2×10^{-4} M neutral solution, while the values obtained in the present study were 3.8 and 3.3 for 10^{-3} and 10^{-2} M solutions, respectively. The fairly good agreement found between the experimental and predicted values, along with checks between data from other laboratories and those from the current investigation indicate that the reactions proposed are reasonable.

4.4 Effect of pH

4.4.1 G_{red}

It has been suggested[69,80] that the solvated electron may react with the thiol to produce hydrogen sulfide, shown in reaction (54). Such a reaction would compete with the reaction:

$$e^-_{aq} + H^+ \longrightarrow H \qquad (55)$$

which, in acid solution, is considered to convert the solvated electrons to hydrogen atoms. Since the rate constant for the latter reaction is very high,[10] it can be assumed that in the pH range 0 - 1 virtually all solvated electrons will be converted to hydrogen atoms before reacting with solutes present at concentrations of 10^{-2} M or less. Hydrogen atoms are therefore taken as the major reducing species under these conditions, and G_H (or G_{red}) is considered to be close to 3.65.[11,81] From Figure 15, for 10^{-2} M cysteine solutions, $G(H_2) = 3.35$ and $G(H_2S) = 0.78$ at pH 0. Thus the sum $G(H_2) + G(H_2S) = 4.13 \pm 0.37$ and if $G^M_{H_2}$ is assumed to be 0.40*, then we find $G_{red} = G(H_2S) + G(H_2) - G^M_{H_2}$ equal to 3.73 ± 0.37. This value is in fair agreement with the accepted value at this pH.

If reaction (43) is the only process giving rise to hydrogen sulfide in this pH range, then

$$k_{37}/k_{43} = \frac{G(H_2) - G^M_{H_2}}{G(H_2S)}$$

which is equal to 3.8 for 10^{-2} M cysteine solutions. Navon and Stein[79] have recently studied the reactions of hydrogen atoms from a discharge tube with cysteine in aqueous solution. They found a value of k_{37}/k_{43} of 7.8 for 1.4×10^{-4} M solutions at 5°C. This is in fair agreement with the earlier result of ~9 obtained by Littman *et al*[68] using the same method but with more concentrated solutions.

Apart from the different sources of hydrogen atoms, the major

*see Appendix I

difference between the discharge tube work and the current investigation is the lower temperature employed by the former. An attempt was made by other workers in this laboratory to determine whether temperature had an effect on the radiolytic value of the ratio k_{37}/k_{43}. Hydrogen and hydrogen sulfide from 10^{-3} M cysteine solutions were determined at pH values of 0, 1 and 6 at 0° C. Yield-dose plots for hydrogen and hydrogen sulfide formation at 0° C are shown in Figure 18. They are linear with dose. A value of the ratio k_{37}/k_{43} at 0° C was determined at pH 0 using the expression given on page 90, and was found to be 8.6. It would appear from this preliminary study that there is a temperature effect on the radiolytic ratio k_{37}/k_{43}.

As shown by the uppermost series of points in Figure 15, the sum of $G(H_2) + G(H_2S)$ is equal to approximately 3.75 in the pH range 6 to 3. At pH 2.5 there is a sudden increase, reaching a limiting value of 4.13 in the pH range 1 - 0. If $G^M_{H_2}$ is taken as 0.40, and is assumed to be independent of pH in the region 6 to 3, then $G_{red} = 3.35$. Subtracting $G_{e^-_{aq}} = 2.75$, one finds $G_H = 0.60$, which is in excellent agreement with the value observed in this pH range with other organic solutes (0.6 ± 0.1).[11] Since $G(H_2)$, the observed hydrogen yield, is 1.10 at pH 6 and taking $G^M_{H_2}$ as 0.40, it would appear that essentially all of the species H forms hydrogen, and that the solvated electron is responsible for the hydrogen sulfide yields in neutral solution. In other words, it seems that the radiolytic ratio k_{37}/k_{43} is pH dependent as well as temperature dependent.

Figure 18

Figure 18

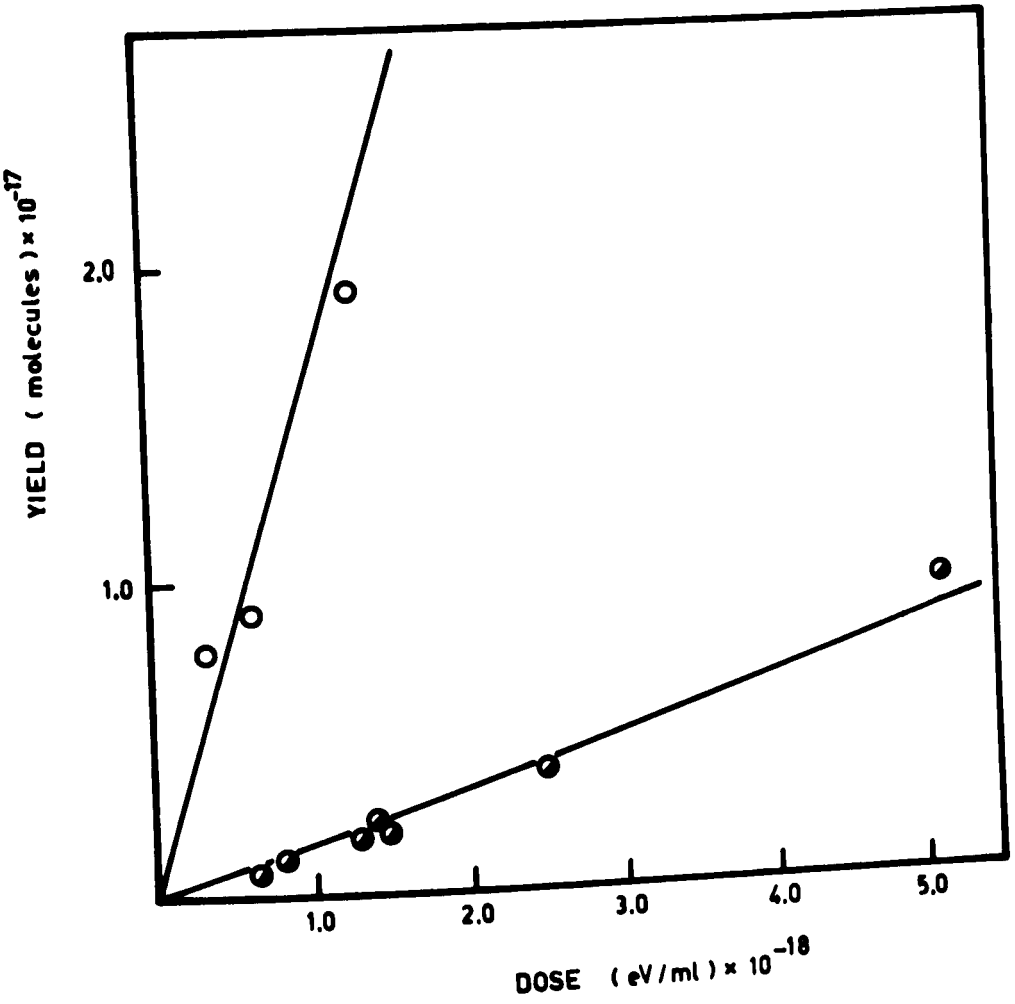
Yields of Hydrogen and Hydrogen Sulfide as a Function of Dose at 0° C

[Cysteine] = 10^{-3} M

pH = 0

o -- Hydrogen

● -- Hydrogen Sulfide


YIELD (molecules) × 10^-17
2.0
1.0
1.0
2.0
3.0
4.0
5.0
DOSE (eV / ml) × 10^-18

It is apparent from Figure 15 that G_{red} must increase by about 0.4 units for 10^{-2} M solutions as the pH is varied from 6 to 0. This increase is in agreement with earlier work concerning the effect of pH on the radiolysis of water (see the section on the radiolysis of water in Introduction).[12,18,44] The increase in G_{red} generally starts at ∿pH 3.5 and reaches a limiting value at pH 1.5.

It may be concluded that the yields of hydrogen and hydrogen sulfide, expressed as G-values, from the radiolysis of 10^{-2} M cysteine solutions correlate with the literature values of G_{red} in both acid and neutral conditions. This agreement makes it reasonable to assume that in 10^{-2} M solutions over the pH range 0 - 6, reactions (37), (43), (54) and (55) are the major reactions of the solvated electron and hydrogen atom. Apart from the formation of molecular hydrogen, these reactions may be regarded as being responsible in the main for the production of hydrogen and hydrogen sulfide.

4.4.2 Yields of hydrogen sulfide, alanine, and hydrogen

If, in neutral solutions, reaction (54) is the primary process leading to formation of hydrogen sulfide:

$$e^-_{aq} + RSH \longrightarrow R + SH^- \qquad (54)$$

and if it is followed by reaction (47)

$$R + RSH \longrightarrow RH + RS \qquad (47)$$

then the yield of RH (alanine) would be expected to be equal to that of

hydrogen sulfide. This was found to be so by Markakis and Tappel,[65] who observed $G(H_2S) = G(\text{Alanine}) = 2.9$ at pH 1.6 for 0.1 M cysteine solutions. Figure 10 shows the dose dependence of both these products for 10^{-3} and 10^{-2} M solutions at neutral pH. Figure 11 shows only the yield of alanine as a function of dose at acid pH. For 10^{-2} M solutions the variation with pH is shown in Figure 15. Within experimental error, the pH dependence of the yields of hydrogen sulfide and alanine are identical. At pH 0, G(Alanine) = 0.86 ± 0.10 while $G(H_2S) = 0.78 \pm 0.8$; in neutral solutions G(Alanine) is equal to 2.47 ± 0.14 and $G(H_2S) = 2.57 \pm 0.25$. For 10^{-3} M solutions, the alanine yields tend to be higher than the hydrogen sulfide yields. The relative rate constants for reaction of solvated electrons with alanine and hydrogen sulfide have both been determined recently,[27,72] ($k_{e^-_{aq}} + \text{alanine} \sim 5 \times 10^6\ M^{-1}\ sec^{-1}$; $k_{e^-_{aq}} + \text{hydrogen sulfide} \sim 1 \times 10^{10}\ M^{-1}\ sec^{-1}$). It is clear that hydrogen sulfide is much more susceptible to radical attack than is alanine. As discussed earlier, at low solute concentrations there will be a competition between products and solute for the reactive intermediates from water. As the cysteine concentration is increased, hydrogen sulfide should be protected and $G(H_2S)$ should become dose and concentration independent. Figure 12 illustrates the latter point. Theoretically, the yields based on the equations previously described should be identical. The reasonable agreement between G(Alanine) and $G(H_2S)$ at higher solute concentrations tends to support the conclusions that these reactions occur under the conditions of this study.

The fate of the solvated electron in an aqueous solution

of cysteine has been discussed[69,80] in terms of the following competing reactions:

$$e^-_{aq} + RSH \longrightarrow R + SH^- \quad (54)$$

$$SH^- + H^+ \longrightarrow H_2S \quad (45)$$

$$e^-_{aq} + H^+ \longrightarrow H \quad (55)$$

Reaction (55) converts the solvated electron to a hydrogen atom, with a known rate constant of 2.2×10^{10} M^{-1} sec^{-1}.[11] As the pH of the solution is decreased, the competition illustrated above should be manifested in drastic changes in the relative amounts of hydrogen and hydrogen sulfide formed. This was found to be the case (see Figure 15). The downward trend of $G(H_2S)$ over the pH range corresponding to the increase in $G(H_2)$ provides strong support for the competition between reactions (54) and (55).

On the basis of the reactions proposed it can be shown that:*

$$\text{(iii)}\quad G(H_2) = \left\{G_H + G_{e^-_{aq}}\left(\frac{k_{55}[H^+]}{k_{54}[RSH] + k_{55}[H^+]}\right)\right\}\frac{k_{37}}{k_{37} + k_{43}} + G^M_{H_2}$$

$$\text{(iv)}\quad G(H_2S) = \left\{G_H + G_{e^-_{aq}}\left(\frac{k_{55}[H^+]}{k_{54}[RSH] + k_{55}[H^+]}\right)\right\}\frac{k_{43}}{k_{37} + k_{43}} + G_{e^-_{aq}}\left(\frac{k_{54}[RSH]}{k_{54}[RSH] + k_{55}[H^+]}\right)$$

The rate constants refer to the reactions defined earlier.

*see Appendix II

In order to calculate expected yields, a knowledge of the ratio k_{37}/k_{43} is required. From a preliminary study carried out in this laboratory, it seems that the radiolytic value of this ratio may be temperature dependent. Therefore, the value obtained in strongly acid solutions at room temperature by the author was used to calculate the expected yields. The ratio k_{54}/k_{55} was determined from the literature values for reactions of the solvated electron with cysteine and acid.[26,27] Figure 19 shows the calculated yields (solid lines) of hydrogen and hydrogen sulfide for 10^{-2} M cysteine solution over the pH range 0 - 6. The experimentally determined G-values are shown by circles. The general correlation which is obtained between the calculated and experimental values indicates that the reactions which have been suggested for the formation of the identified products are reasonable, but the lack of better agreement clearly indicates that the radiolytic processes are not fully understood. Possibly a more complete study of the effects of temperature and pH on the ratio k_{37}/k_{43} would result in closer correlation.

4.5 Competition Kinetics

At pH 7, the $G(H_2S)$ value of $\sim$ 2.5 for 10^{-2} M cysteine solutions was thought to arise from two sources, a small part from reaction (43)

$$H + RSH \longrightarrow H_2S + R \qquad (43)$$

due to the reaction of part of the "residual" hydrogen yield, and the majority from reaction (54)

Figure 19

Figure 19

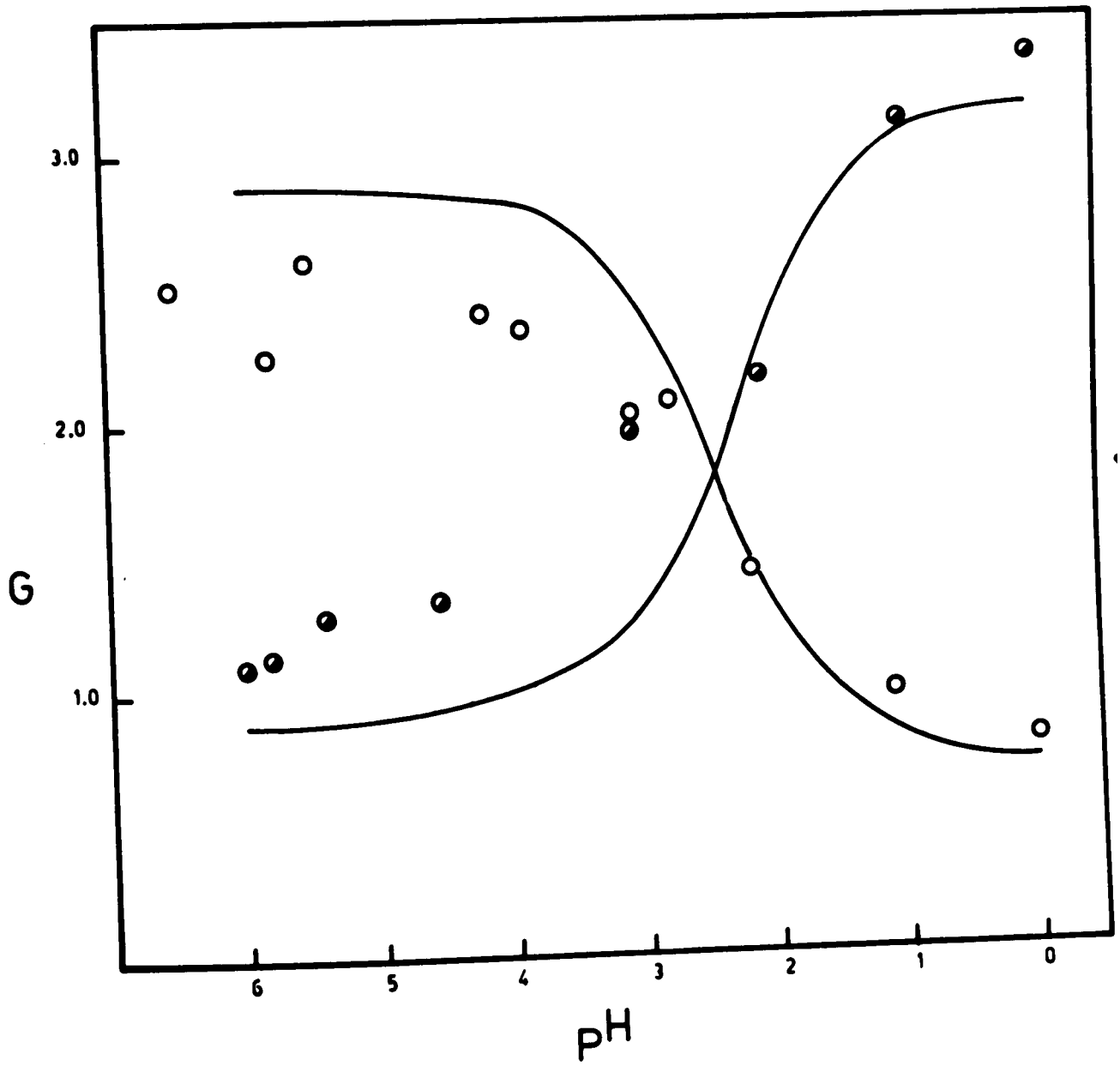
pH Dependence of Experimental and Calculated G-values for Hydrogen and Hydrogen Sulfide

[Cysteine] = 10^{-2} M

——— -- Calculated G-values

● -- Experimental values of $G(H_2)$

o -- Experimental values of $G(H_2S)$


3.0
2.0
1.0
G
6
5
4
3
2
1
0
pH

$$e^-_{aq} + RSH \longrightarrow SH^- + R \qquad (54)$$

In order to test this hypothesis further, competition kinetics were carried out in which known electron scavengers were added to the cysteine solutions before irradiation and the yield of hydrogen sulfide followed as a function of scavenger concentration. Acetone and nitrate ion were chosen as representative scavengers, due to their very high rate constants for reaction with solvated electrons.[26] $G(H_2S)$ was determined from the initial slopes of the yield-dose plots for each concentration of scavenger. On the basis of the competition between RSH and acetone, represented in the equations:

$$e^-_{aq} + RSH \longrightarrow SH^- + R \qquad (54)$$

$$e^-_{aq} + \text{acetone} \longrightarrow \text{products} \qquad (57)$$

the following relationship should hold*

$$(v) \quad \Delta G(H_2S) = G_{e^-_{aq}} \left\{ \frac{k_{57}[\text{acetone}]}{k_{54}[RSH] + k_{57}[\text{acetone}]} \right\}$$

$\Delta G(H_2S)$ refers to the difference in $G(H_2S)$ between that obtained with added acetone and that with no added acetone. The above expression may be rearranged to give

$$(vi) \quad \frac{1}{\Delta G(H_2S)} = \frac{1}{G_{e^-_{aq}}} \left\{ 1 + \frac{k_{54}[RSH]}{k_{57}[\text{acetone}]} \right\}$$

*see Appendix II

and a plot of $\frac{1}{\Delta G(H_2S)}$ versus [RSH]/[acetone] should be linear with a slope of $\frac{1}{G_{e^-_{aq}}}\left\{\frac{k_{54}}{k_{57}}\right\}$ and intercept = $\frac{1}{G_{e^-_{aq}}}$. The data for added acetone are presented in accordance with expression (vi) in Figure 20. The results of separate experiments with added nitrate ion as electron scavenger

$$e^-_{aq} + NO_3^- \longrightarrow \text{products} \qquad (58)$$

also fitted expression (vi) (see Figure 21).

From these plots (Figures 20 and 21) the following ratios of rate constants were obtained:

$$\frac{k_{54}}{k_{57}} = \frac{k_{e^-_{aq}} + RSH}{k_{e^-_{aq}} + \text{acetone}} = 1.44$$

$$\frac{k_{54}}{k_{58}} = \frac{k_{e^-_{aq}} + RSH}{k_{e^-_{aq}} + NO_3^-} = 1.03$$

From the known rate constants for the reactions of the solvated electron with acetone and nitrate ion,[26] the rate constant for reaction (54) may be calculated. For the acetone studies,

$$k_{54} = 8.5 \times 10^9\ M^{-1}\ sec^{-1}$$

and for the nitrate studies,

$$k_{54} = 1.10 \times 10^{10}\ M^{-1}\ sec^{-1}$$

Figure 20

Figure 20

Kinetic Plot for the Addition of Acetone to Irradiated Cysteine Solutions

A plot of $\frac{1}{\Delta G(H_2S)}$ versus [cysteine]/[acetone]

[Cysteine] = 10^{-2} M

pH = 5

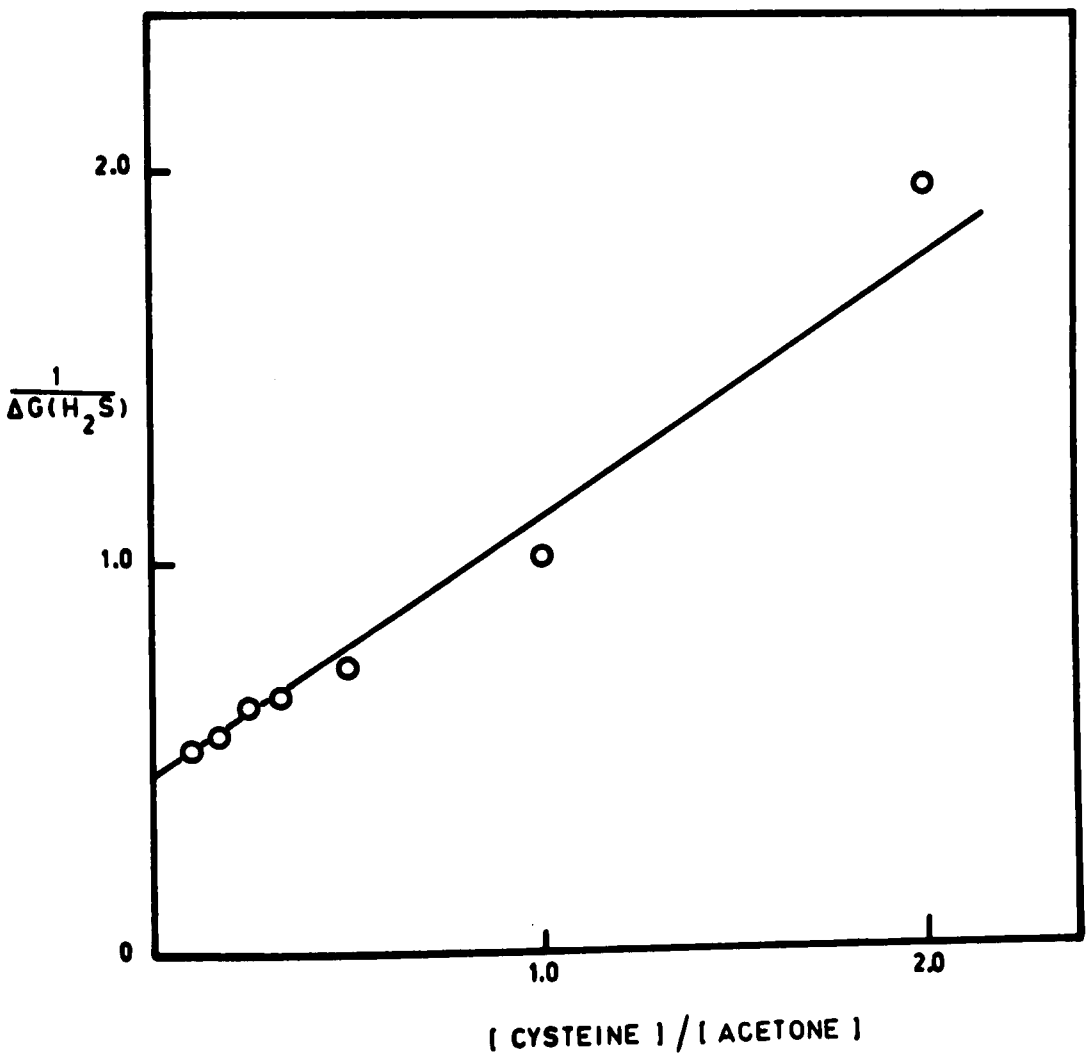

2.0
1.0
0
1.0
2.0
$\frac{1}{\Delta G(H_2S)}$
[CYSTEINE] / [ACETONE]

Figure 21

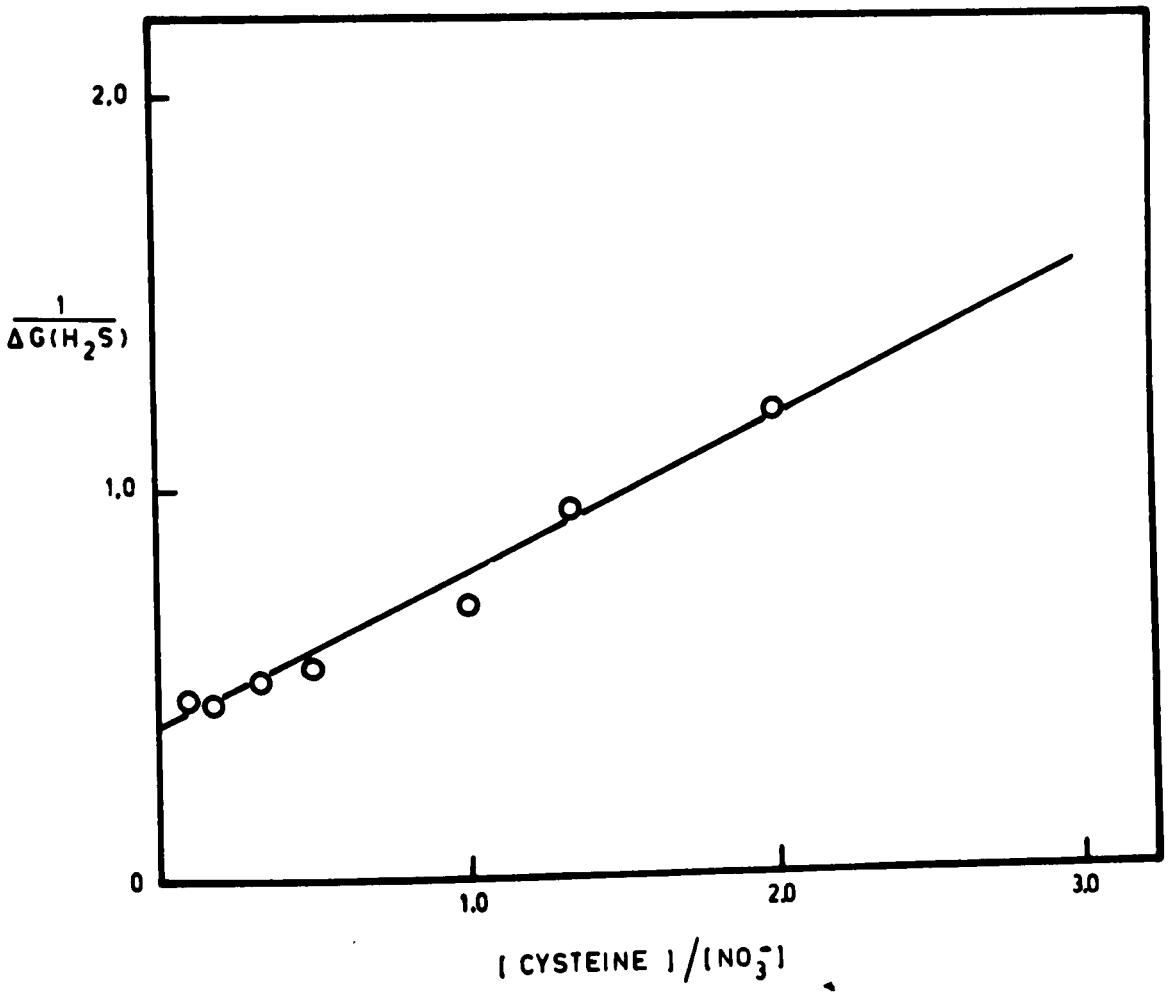
Figure 21

Kinetic Plot for the Addition of Nitrate Ion to Irradiated Cysteine Solutions

A plot of $\frac{1}{\Delta G(H_2S)}$ versus [cysteine]/[NO_3^-]

[Cysteine] = 10^{-2} M

pH = 5


2.0
1.0
0
$\frac{1}{\Delta G(H_2S)}$
1.0
2.0
3.0
[CYSTEINE] / [NO_3^-]

Since it is expected from reaction (54) and (47) that the yields of alanine should equal those of hydrogen sulfide, the effect of electron scavengers on the two yields should also be similar. Although this matter was not investigated in any detail, a preliminary study was carried out using acetone and 10^{-3} M cysteine. A decrease in G(Alanine) corresponding, approximately, to that found in $G(H_2S)$ for the same concentration of added scavenger was noted. This matter warrants further investigation.

During the course of this work other research groups have published evidence of a reaction between cysteine and the solvated electron. Trumbore *et al*[69] have carried out competition experiments using acetone and nitrate ion at lower cysteine concentrations than the present study; Hart *et al*[82] at the Argonne National Laboratory have determined the absolute value of $k_{e^-_{aq} + RSH}$ in alkaline and neutral conditions; and Braams[27] also using the pulsed radiolysis technique has obtained an absolute value for the rate constant. Table III shows the rate constants for reactions of the solvated electron with cysteine from the present investigation along with those obtained for cysteine and cystine by other workers. From the last column of the table it may be seen that the values of k_{54} for cysteine in neutral or acid solution lie in the range $(4 - 11) \times 10^9$ M^{-1} sec^{-1}, with the absolute rate determination most recently obtained by Braams at 8.7×10^9 M^{-1} sec^{-1}. The results from the present project are, within experimental error, close to this. Although the variation between the results of

TABLE III

Thiol	Concentration (M)	pH	Scavenger	k_{54} (M^{-1} sec^{-1})	Ref.
Cysteine	7×10^{-4}	neutral	nitrate	4.4×10^{9}	(69)
	7×10^{-4}	neutral	acetone	5.3×10^{9}	(69)
	not known	neutral	absolute	6.0×10^{9}	(26)
	not known	alkaline	absolute	10^{6}-10^{7}	(26)
	10^{-2}	neutral	absolute	8.7×10^{9}	(27)
	10^{-2}	alkaline	absolute	7.5×10^{7}	(27)
	10^{-2}	neutral	nitrate	11.0×10^{9}	†
	10^{-2}	neutral	acetone	8.5×10^{9}	†
Methyl Mercaptan	6.5×10^{-3}	acid	H^{+}	23.2×10^{9}	†
	5×10^{-2}	acid	H^{+}	16.7×10^{9}	†
Mercapto-ethanol	10^{-2}	acid	H^{+}	13.3×10^{9}	†
Cystine	1.5×10^{-2}	neutral	absolute	13×10^{9}	(27)

† present study

this study and those of Trumbore's[69] may be only a reflection of the combined experimental uncertainties, it is of some interest. There is a difference of 10 in the concentrations of cysteine used in the two competition studies. This may be significant, in view of the fact that as the cysteine concentration decreases, $G(H_2S)$ was found to be lower than expected. However, the effect of concentration on the ratio k_{54}/k_{57} has not been investigated in detail. Such a study might prove worthwhile in that it might indicate whether k_{54} is concentration dependent or not.

The lower values obtained for the rate constant in alkaline solution[26,27] are probably due to ionization of the thiol group ($pK_a = 8.36$) and the consequent negative charge on the sulfur atom. Attack of the solvated electron to cleave the $C\text{-}S^-$ bond and produce $S^=$ is likely to be very slow. In support of this Dale and Davies[67] found a very sharp decrease in the yield of hydrogen sulfide from cysteine and glutathione solutions above pH 7.

4.6 Total Decomposition of Cysteine

It has been shown by other workers[66,69,70,71] that the yields for the disappearance of the thiol group in deaerated cysteine solutions, G(-RSH), are quite sensitive to both pH and initial concentration of cysteine. For example, Trumbore[69] found that in acid solutions, G(-RSH) was $\sim$4 for 2×10^{-4} M, and $\sim$11 for 4×10^{-3} M cysteine. In neutral solutions, the values obtained were 8 and 20 molecules per 100 eV, respectively. Packer[66] found G(-RSH) = 6.2

for 4.4×10^{-3} M solutions at pH 4. The data from Matsuura's study[71] indicate that in acid solution G(-RSH) has values of 5.4 and 7.2 for 10^{-3} and 10^{-2} M solutions respectively, while in neutral solutions G(-RSH) = 6.4 for both concentrations. These data are summarized in Table IV.

To account for the data obtained in the present investigation and those from earlier studies, the following mechanism has been postulated:

$$H_2O \longrightarrow H_2 + H_2O_2 + H + OH \quad (1)$$

$$RSH + OH \longrightarrow H_2O + RS \quad (33)$$

$$RSH + H \longrightarrow H_2 + RS \quad (37)$$

$$RSH + H \longrightarrow H_2S + R \quad (43)$$

$$RSH + R \longrightarrow RH + RS \quad (47)$$

$$RS + RS \longrightarrow RSSR \quad (52)$$

$$e^-_{aq} + RSH \longrightarrow R + SH^- \quad (54)$$
$$\qquad\qquad\qquad\qquad\qquad\quad \downarrow \longrightarrow H_2S \quad (45)$$

$$e^-_{aq} + H^+ \longrightarrow H \quad (55)$$

On the basis of these reactions it may be shown that, in acid solutions,

$$G(-RSH) = G_{OH} + G(H_2) - G^M_{H_2} + G(H_2S) + G(\text{Alanine})$$

For 10^{-2} M cysteine solutions, G(-RSH) = 7.5, and for 10^{-3} M solutions,

TABLE IV

Cysteine (M)	pH	G(-RSH)	Reference
2×10^{-4}	acid	4	(69)
4×10^{-3}	acid	11	(69)
10^{-3}	acid	5.4	(71)
10^{-2}	acid	7.2	(71)
4.4×10^{-3}	4	6.2	(66)
2×10^{-4}	neutral	8	(69)
4×10^{-3}	neutral	20	(69)
10^{-3}	neutral	6.4	(71)
10^{-2}	neutral	6.4	(71)

G(-RSH) = 7.4. In the latter case, G(Alanine) was used in place of $G(H_2S)$, since it is somewhat more reliable. Another worker in this laboratory has recently obtained a value for G(-RSH) of 7.6 for 10^{-3} M cysteine in acid solution[77] and ~8.0 for 10^{-2} M solutions. This value must be regarded as tentative until additional quantitative studies have been carried out. The project, utilizing the amino acid analyzer, is currently being pursued. The calculated value is also in reasonable agreement with that obtained by Matsuura.[71]

In neutral solutions, it has been shown[70] that a reaction between hydrogen peroxide and cysteine occurs:

$$2\ RSH + H_2O_2 \longrightarrow RSSR + 2\ H_2O \qquad (51)$$

Also, from the present study, it was noted that in neutral solution, all hydrogen atoms present reacted <u>via</u> reaction (37). Therefore,

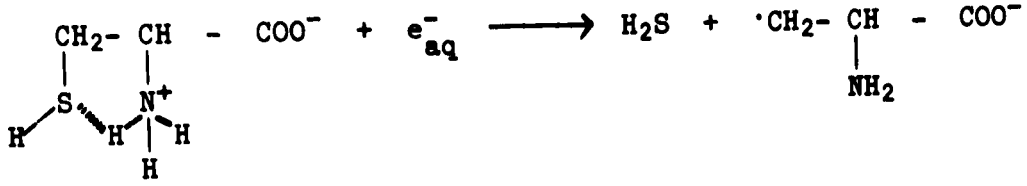
$$G(-RSH) = G_{OH} + G(H_2) - G^M_{H_2} + G(H_2S) + 2\,G^M_{H_2O_2} + G(\text{Alanine})$$

For 10^{-2} M solution at neutral pH, G(-RSH) should therefore be 9.3, while for 10^{-3} M solutions, G(-RSH) should be 8.6.

4.7 Comparison with Other Related Thiols

It was noted by early workers in the field that those thiols most active as protective agents contain either a thiol or disulfide group and an amino group. Maximal protection is obtained when these two groupings are separated by only two or three carbon atoms. It is

possible that they may exist as hydrogen-bonded conformers, as shown in the figure below for cysteine (I):



I

Such a conformer might undergo a concerted reaction to eliminate hydrogen sulfide in one step, in contrast to removal of SH^- ion (reaction (54)) and its subsequent reaction with H^+. If this were the case, then reactivity towards the solvated electron might be expected to be a special property of the 2,3-aminothiols and not of thiols in general. It was essential that this be tested, since in many naturally-occurring thiols the amino group is not free but involved in a peptide linkage. Therefore, studies were made in this laboratory of the pH dependence of various products from the radiolysis of two simpler thiols - methyl mercaptan (V) and 2-mercaptoethanol (VI). The more recent results have not

CH_3SH

V

$HS - CH_2 - CH_2 - OH$

VI

been subjected to detailed analysis. Since they have a direct bearing on the interpretation of the cysteine results, they will now be discussed in considerable detail.

Hydrogen yields from mercaptan solutions in two different

concentration ranges, (5 - 8) x 10^{-3} M and (4 - 6) x 10^{-2} M, have been measured. Over the range of doses used, (0.2 - 3.0) x 10^{18} eV/ml, the specific yields were independent of dose. The dependence of the hydrogen yields expressed as G-values on pH is shown in Figure 22.

For methyl mercaptan, the R radical in reactions (43) and (54) would be a methyl radical. For example

$$H + CH_3SH \longrightarrow H_2S + CH_3 \qquad (43)$$

This should abstract a hydrogen atom from another thiol molecule via reaction (47) to form methane

$$CH_3 + CH_3SH \longrightarrow CH_4 + CH_3S\cdot \qquad (47)$$

Thus, $G(CH_4)$ should be identical with $G(H_2S)$ for methyl mercaptan solutions, in the same way that G(Alanine) is in the cysteine solutions. In all methyl mercaptan experiments, a second non-condensible gas remained after diffusion of the hydrogen through a palladium thimble. This was confirmed by mass spectrometric analysis to be methane. The approximate G-values for methane were determined from the amount of residual gas remaining in the gas analysis apparatus after hydrogen diffusion through the thimble, and are shown, along with $G(H_2)$, for (4 - 6) x 10^{-2} M methyl mercaptan solutions as a function of pH in Figure 22.

It should be noted that in the studies with methyl mercaptan the pH of the solutions was adjusted with sulfuric acid and perchloric acid. The difference in $G(H_2)$ was negligible, and it can

Figure 22

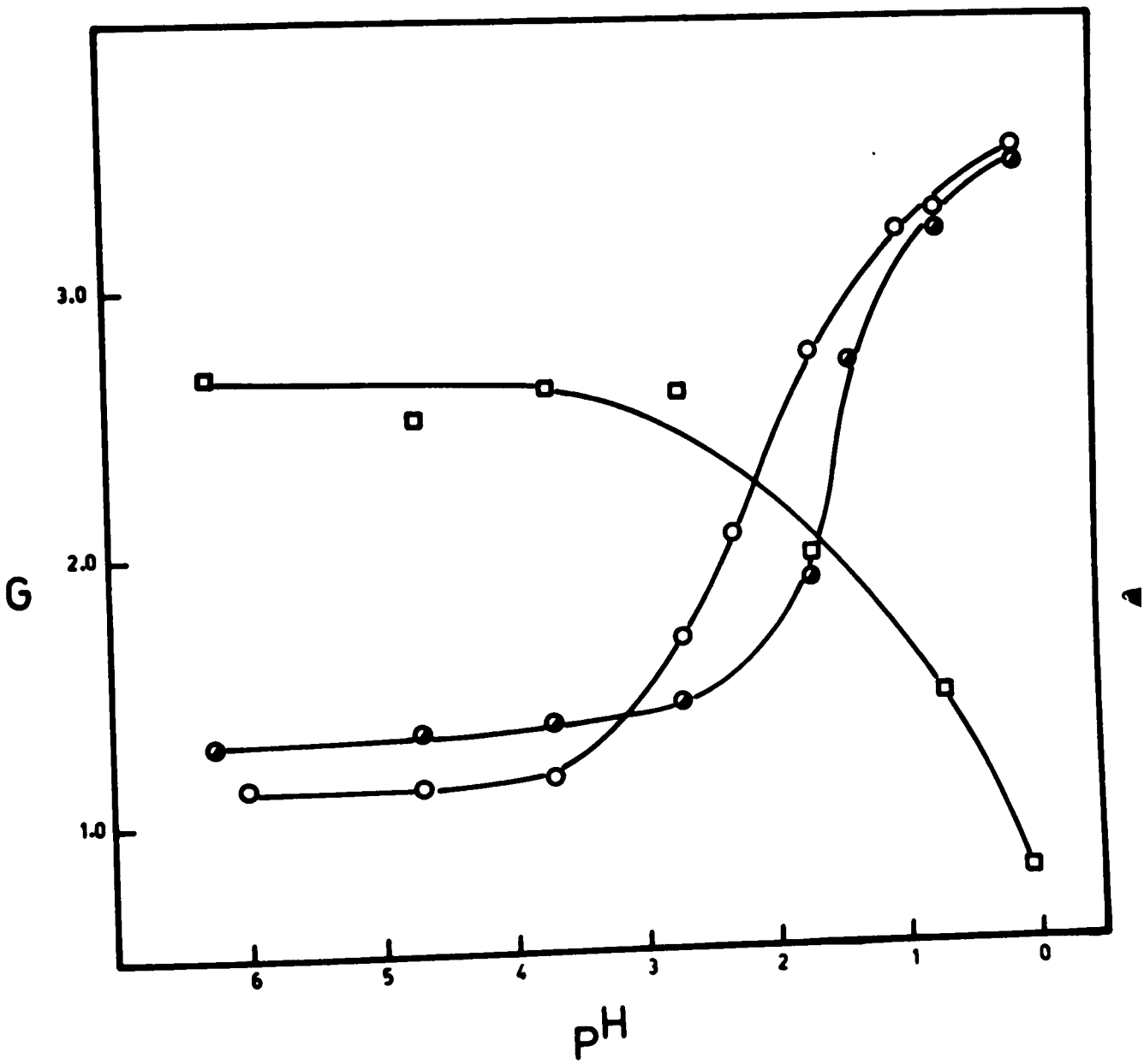
Figure 22

Variation of $G(H_2)$ and $G(CH_4)$ with pH for Irradiated Methyl Mercaptan Solutions

○ -- Hydrogen, Methyl Mercaptan = 6.5×10^{-3}M

● -- Hydrogen, Methyl Mercaptan = 5×10^{-2}M

□ -- Methane, Methyl Mercaptan = 5×10^{-2}M


3.0
2.0
1.0
G
6
5
4
3
2
1
0
pH

be concluded that it is independent of the acid used to vary the pH. It was observed during the course of this project that when sulfuric acid was used to vary the pH with 10^{-3} M cysteine solutions, the hydrogen yields were "low" - i.e. they were somewhat lower than those from perchloric acid solutions of other thiols in the pH range 0 - 1. For this reason, hydrogen yields from cysteine solutions were redetermined using perchloric acid to vary the pH. The new hydrogen yields were found to be higher (G-value of 3.1) than those from the sulfuric solutions (2.5) and in better agreement with the corresponding values for other thiols. The yields from perchloric solutions are shown in Figure 15. It is known that perchlorate ions do not react as readily with free radicals generated in water as do other common anions such as sulfate, nitrate or chloride.[83] Furthermore, perchlorate ions show a smaller tendency to form complexes than do sulfate ions. These two factors might account for the difference in product yields from aqueous solutions of the two acids.

Hydrogen and hydrogen sulfide yields have been determined for aqueous solutions of 2-mercaptoethanol at two concentrations over the dose range (0.4 - 7) x 10^{18} eV/ml. The pH dependence of the yields of these products from 10^{-2} M solutions is shown in Figure 23.

If the data obtained from the radiolysis of methyl mercaptan and 2-mercaptoethanol solutions are analyzed in a similar manner to that from cysteine solutions, G_{red} may be calculated. The results of this calculation are shown in Table V. Those from cysteine solutions are included for comparison. In acid solution, G_{red} is considered to

Figure 23

Figure 23

pH Dependence of $G(H_2)$ and $G(H_2S)$ for 10^{-2} M 2-Mercaptoethanol Solutions

● -- Hydrogen

o -- Hydrogen Sulfide

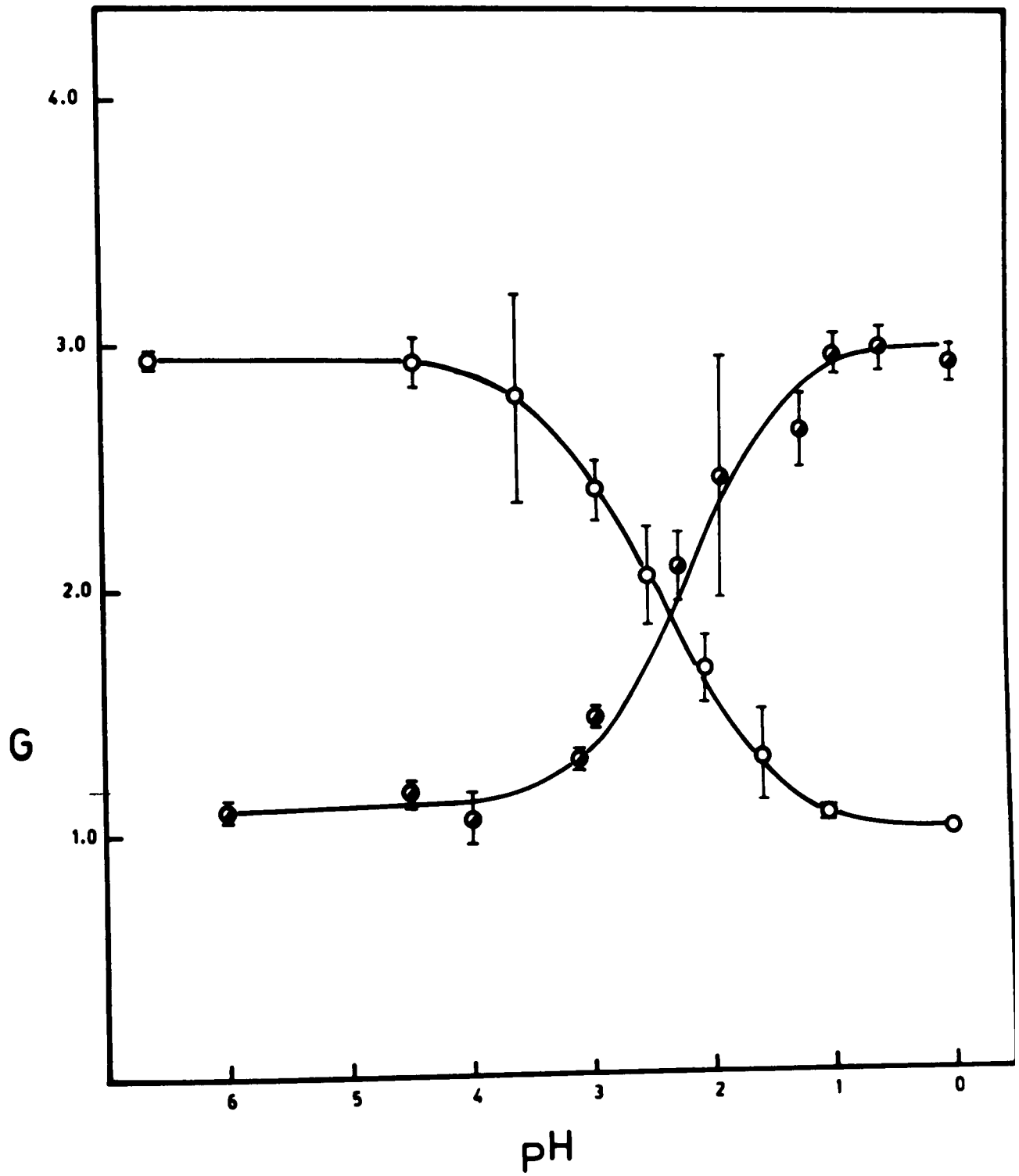

4.0
3.0
2.0
1.0
G
6
5
4
3
2
1
0
pH

TABLE V

Thiol	Concentration	pH	G_{red}	G_H	k_{37}/k_{43}	k_{54}/k_{55}
Cysteine	10^{-2} M	0	3.73	-	3.8	-
Cysteine	10^{-3} M (25°C)	0	3.32	-	4.3	-
Cysteine	10^{-3} M (0°C)	0	3.16	-	8.6	-
2-mercapto-ethanol	10^{-2} M	0	3.58	-	2.6	-
Methyl mercaptan	5×10^{-2} M	0	3.96	-	4.1	-
Cysteine	10^{-2} M	3 - 6	3.35	0.60	-	-
2-mercapto-ethanol	10^{-2} M	3 - 6	3.60	0.85	-	0.61
Methyl mercaptan	5×10^{-2} M	3 - 6	3.50	0.75	-	0.76
Methyl mercaptan	6.5×10^{-3} M	3 - 6	-	-	-	1.05

be 3.65, so that the results from 10^{-2} M mercaptoethanol solutions appear to be in very good agreement with the accepted yield and with the results from cysteine. For 5×10^{-2} M methyl mercaptan solutions, it is apparent that G_{red} is slightly larger than that found for cysteine. Since it has been shown[84] that G_{red} increases slightly with increasing solute concentration, the observed increase in G_{red} in the more concentrated methyl mercaptan solutions is reasonable.

If, in acid solution, all solvated electrons are converted to hydrogen atoms by means of reaction (55), then it should be possible to calculate the ratio of rate constants for reactions (37) and (43) at room temperature using the following expression:

$$\frac{G(H_2) - G^M_{H_2}}{G(H_2S)} = k_{37}/k_{43}$$

Since methane yields should be identical with hydrogen sulfide yields for methyl mercaptan solutions, $G(CH_4)$ may be used in place of $G(H_2S)$. The results of these calculations are also given in Table V.

Figures 22 (for methyl mercaptan) and 23 (for mercaptoethanol) indicate that the pH dependence of $G(H_2)$ is qualitatively similar to that found for cysteine. The results were analyzed by means of expression (vii):

$$\text{(vii)} \quad \frac{1}{\Delta G(H_2)} = \frac{k_{37} + k_{43}}{G_{e^-_{aq}} \times k_{37}} \left\{ 1 + \frac{k_{54}[RSH]}{k_{55}[H^+]} \right\}$$

In this expression $\Delta G(H_2)$ is the increase in hydrogen yield over the

plateau yield observed at higher values of pH, and the rate constants are for the reactions previously defined. A plot of $\frac{1}{\Delta G(H_2)}$ versus $[RSH]/[H^+]$ is generally referred to as a "reciprocal plot". It should take the form of a straight line, with slope = (intercept) $\frac{k_{54}}{k_{55}}$ and intercept = $\frac{k_{37} + k_{43}}{G_{e^-_{aq}} \times k_{37}}$. Similar expressions frequently have been used to obtain estimates of relative rate constants. The values obtained in this instance must be regarded as approximate for two reasons: first, the effect of pH on the ratio k_{37}/k_{43} is not known, and secondly, there is a great deal of uncertainty in the value of $G_{e^-_{aq}}$.

Reciprocal plots for methyl mercaptan at two concentrations are shown in Figure 24 and for mercaptoethanol in Figure 25. For 10^{-2} M solutions the linear relationship makes it possible to determine the ratio k_{54}/k_{55}. The results are given in Table V. From this ratio, and substituting the known value of $k_{55}(2.2 \times 10^{10}\ M^{-1}\ sec^{-1})$, one finds k_{54} for 5×10^{-2} M methyl mercaptan equal to $1.67 \times 10^{10}\ M^{-1}\ sec^{-1}$, and k_{54} for 10^{-2} M mercaptoethanol equal to $1.33 \times 10^{10}\ M^{-1}\ sec^{-1}$.

The high rate constants obtained in this laboratory for the three thiols under investigation indicate that the sulfhydryl group by itself can react with the solvated electron - i.e. the amino group is not essential for such a reaction to occur.

4.8 Implications for Radiation Biology

Most attempts to explain the inactivation of the molecules in the living cell by direct or indirect action have involved reactive species formed primarily from water molecules. The disulfide bond, which

Figure 24

Figure 24

Kinetic Plot of the Effect of Acid on the Hydrogen Yield from Irradiated Methyl Mercaptan Solutions

A plot of $\frac{1}{\Delta G(H_2)}$ versus $[CH_3SH]/[H^+]$

o -- 6.5×10^{-3} M methyl mercaptan solution

● -- 5×10^{-2} M methyl mercaptan solution

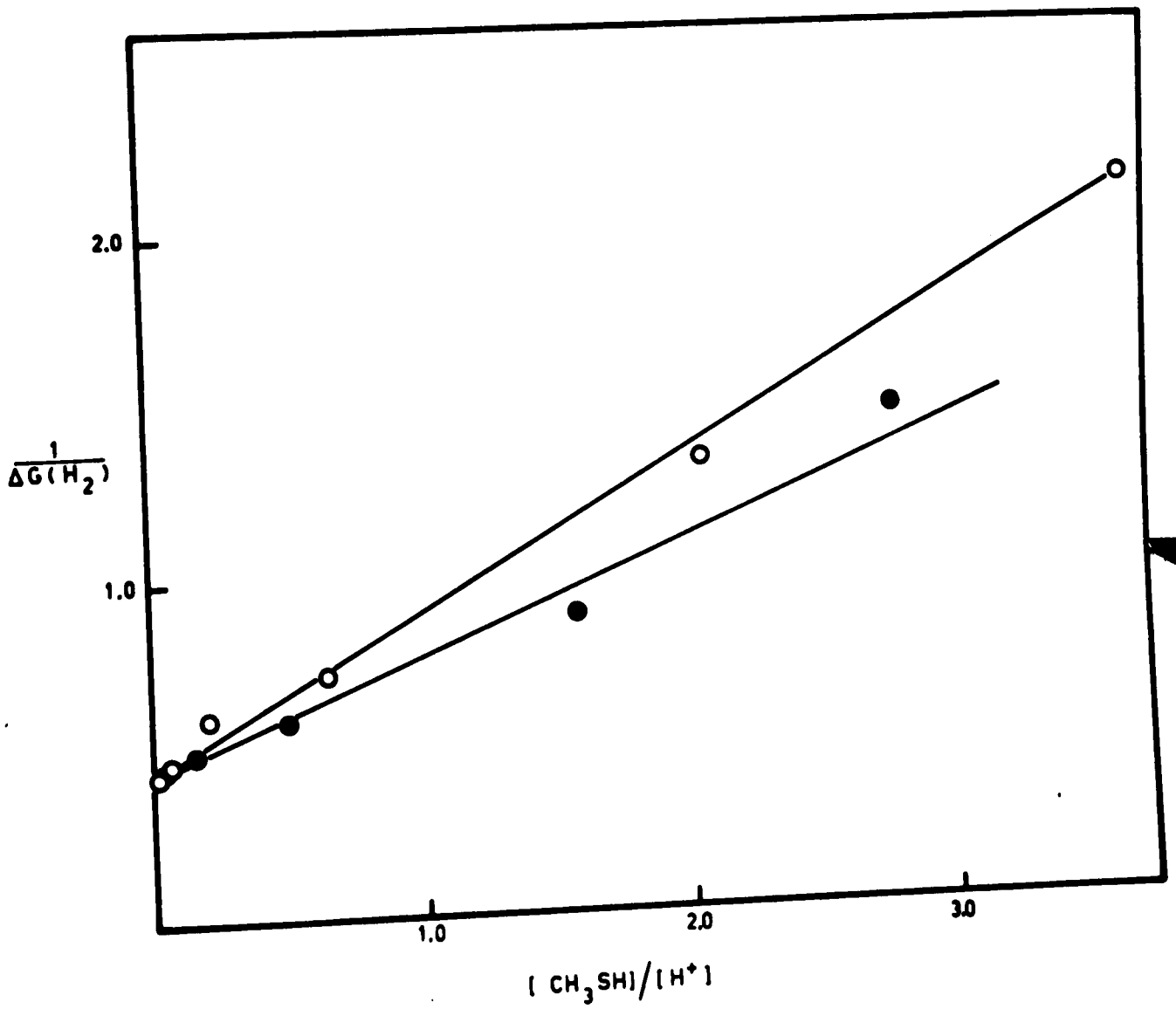

2.0
1.0
$\frac{1}{\Delta G(H_2)}$
1.0
2.0
3.0
$[CH_3SH]/[H^+]$

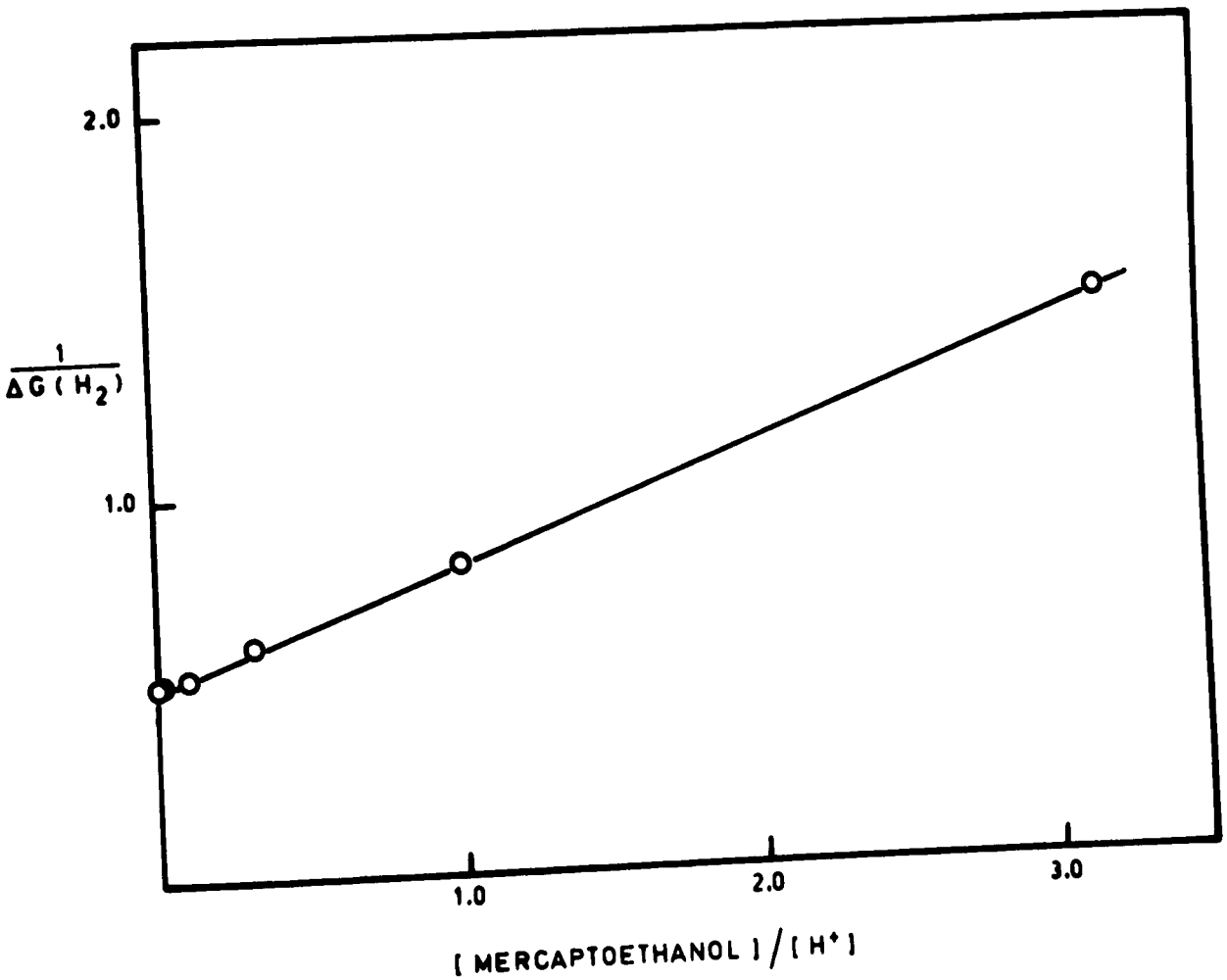
Figure 25

Figure 25

Kinetic Plot of the Effect of Acid on the Hydrogen Yield from Irradiated 2-Mercaptoethanol Solutions

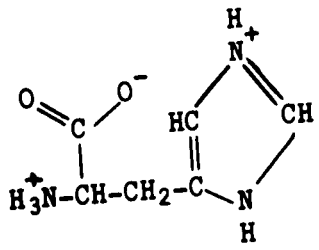
A plot of $\frac{1}{\Delta G(H_2)}$ versus [mercaptoethanol]/[H^+]

[2-Mercaptoethanol] = 10^{-2} M


2.0
1.0
1/ΔG(H2)
1.0
2.0
3.0
[MERCAPTOETHANOL] / [H+]

is more radiosensitive than the sulfhydryl bond,[27,55] is of great importance in the conservation of the secondary and tertiary structure of many proteins. However, because of its location between peptide chains, this bond is more difficult to approach, and therefore might be less reactive in the native protein than in cystine.

Pulsed radiolysis experiments[27] indicate that several amino acids react very rapidly with the solvated electron. In aqueous deaerated solutions of proteins these residues could be a vulnerable site for attack by the solvated electron if it can approach the reactive acid. This should be the case for histidine (VII) and cysteine:



VII

which are often located at sites accessible to diffusing free radicals.

It is apparent from this and other studies[70] that cysteine, one of the better protective agents, is quite effective as a scavenger for hydroxyl radicals and solvated electrons, the two major reactive intermediates known to exist in neutral irradiated aqueous solutions. It seems reasonable to consider the possibility that many of the reactive intermediates are scavenged before they are able to attack biologically important molecules. Howard-Flanders[85] had suggested this possibility as one explanation for the protection afforded E. Coli B/r in aqueous suspensions by mercaptoethanol.

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APPENDIX I

The fact that radical and molecular yields vary with solute concentration has been known for some time.[81,86] The effects of solutes on $G^M_{H_2}$ is largely related to the rate constants for their reaction with the solvated electron.[87] Since $G^M_{H_2}$ from thiol solutions cannot be measured directly, its value for various concentrations of the thiols used in this study has been estimated from the value of $G^M_{H_2}$ obtained in nitrite solutions of similar concentrations.[86] The rate constants for reaction between nitrite ion and solvated electrons is not very different from that reported in this study for the thiols. Hence from plots of $G^M_{H_2}$ versus $[NO_2^-]$ one may estimate $G^M_{H_2}$ = 0.42, 0.38 and 0.28 for 10^{-3}, 10^{-2} and 10^{-1} M cysteine solutions, respectively.

The depeletion of molecular yields at higher solute concentrations is normally accompanied by an increase in radical yields.[84] Thus in the more concentrated solutions of cysteine and methyl mercaptan one expects G_{red} to increase above 3.65. This was in fact found to be the case. Also it may be noted that G(Cystine) was 3.8 instead of 3.3 as predicted by the yields for dilute netural solutions.

APPENDIX II

Derivation of kinetic expressions for $G(H_2)$ and $G(H_2S)$ from irradiated thiol solutions in the absence and presence of added electron scavengers

Considering the following reactions:

$$RSH + OH \longrightarrow H_2O + RS \qquad (33)$$

$$RSH + H \longrightarrow H_2 + RS \qquad (37)$$

$$RSH + H \longrightarrow H_2S + R \qquad (43)$$

$$RSH + R \longrightarrow RH + RS \qquad (47)$$

$$RS + RS \longrightarrow RSSR \qquad (52)$$

$$e^-_{aq} + RSH \longrightarrow R + SH^- \longrightarrow H_2S \qquad (54)$$

$$e^-_{aq} + H^+ \longrightarrow H \qquad (55)$$

If it is assumed that the observable hydrogen yield, $G(H_2)$, arises from molecular hydrogen from the spurs and from hydrogen atom attack on the thiol, RSH, then the following relationships may be written:

$G(H_2)$ = H_2 from spurs + H_2 from H atom attack on RSH

= $G^M_{H_2}$ + (total number of H atoms per 100 eV) x (fraction of H atoms leading to H_2)

$$G(H_2) = G^M_{H_2} + \left\{ G_H + G_{e^-_{aq}} \left(\text{fr. } e^-_{aq} \rightarrow H \right) \right\} \left\{ \text{fr. } H \longrightarrow H_2 \right\}$$

$$\text{(iii)}\quad G(H_2) = G^{M}_{H_2} + \left\{ G_H + G_{e^-_{aq}} \left(\frac{k_{55}[H^+]}{k_{54}[RSH] + k_{55}[H^+]} \right) \right\} \frac{k_{37}}{k_{37} + k_{43}}$$

(iv) $G(H_2S)$ = H_2S from H atom attack on RSH + H_2S from e^-_{aq} attack on RSH

= (total number of H atoms per 100 eV) x (fr. H $\longrightarrow$ H_2S)

$$+ G_{e^-_{aq}} (\text{fr. } e^-_{aq} \longrightarrow H_2S)$$

$$= \left\{ G_H + G_{e^-_{aq}} \left(\begin{matrix} \text{fr. } e^-_{aq} \\ \longrightarrow H \end{matrix} \right) \right\} \left\{ \begin{matrix} \text{fr. H} \\ \longrightarrow H_2S \end{matrix} \right\} + G_{e^-_{aq}} (\text{fr. } e^-_{aq} \longrightarrow H_2$$

$$= \left\{ G_H + G_{e^-_{aq}} \left(\frac{k_{55}[H^+]}{k_{54}[RSH] + k_{55}[H^+]} \right) \right\} \left\{ \frac{k_{43}}{k_{37} + k_{43}} \right\} + G_{e^-_{aq}} \left(\frac{k_{54}[RSH]}{k_{54}[RSH] + k_{55}[H^+]} \right)$$

For added electron scavengers, for example, acetone:

$$e^-_{aq} + \text{acetone} \longrightarrow \text{products} \qquad (5$$

$$G(H_2S) = \left\{ G_H + G_{e^-_{aq}} \left(\frac{k_{55}[H^+]}{k_{54}[RSH] + k_{55}[H^+] + k_{57}[\text{acetone}]} \right) \right\} \frac{k_{43}}{k_{37} + k_{43}} + G_{e^-_{aq}} \left(\frac{k_{54}[RSH]}{k_{54}[RSH] + k_{55}[H^+] + k_{57}[\text{acetone}]} \right)$$

Since, at pH = 6, $[H^+]$ is small, neglect terms involving $k_{55}[H^+]$

$$\therefore \quad G(H_2S) = G_H \left(\frac{k_{43}}{k_{37} + k_{43}} \right) + G_{e^-_{aq}} \left(\frac{k_{54}[RSH]}{k_{54}[RSH] + k_{57}[\text{acetone}]} \right) \qquad (i$$

When [acetone] = 0,

$$G(H_2S) = G_H \left(\frac{k_{43}}{k_{37} + k_{43}} \right) + G_{e^-_{aq}} \qquad ($$

Subtracting (a) from (b) gives

$$\Delta G(H_2S) = G_{e^-_{aq}} - G_{e^-_{aq}} \left(\frac{k_{54}[RSH]}{k_{54}[RSH] + k_{57}[acetone]} \right)$$

and rearranging:

$$\Delta G(H_2S) = G_{e^-_{aq}} \left\{ 1 - \frac{k_{54}[RSH]}{k_{54}[RSH] + k_{57}[acetone]} \right\}$$

$$\text{(v)} \quad \Delta G(H_2S) = G_{e^-_{aq}} \left\{ \frac{k_{57}[acetone]}{k_{54}[RSH] + k_{57}[acetone]} \right\}$$

$$\text{(vi)} \quad \frac{1}{\Delta G(H_2S)} = \frac{1}{G_{e^-_{aq}}} \left\{ 1 + \frac{k_{54}[RSH]}{k_{57}[acetone]} \right\}$$

Therefore, a plot of $\frac{1}{\Delta G(H_2S)}$ versus [RSH]/[acetone] should be linear with a slope of $\frac{1}{G_{e^-_{aq}}} \left(\frac{k_{54}}{k_{57}} \right)$ and an intercept = $\frac{1}{G_{e^-_{aq}}}$.

The expression used for analyzing data from experiments with added nitrate ion may be derived in a similar way.