JAERI-M
94-070

RADIATION PROTECTIVE EFFECTS OF CYSTEAMINE AND GLUTATHIONE ON FOUR NUCLEOBASES AND ASCORBIC ACID IN AQUEOUS SOLUTION

March 1994

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編集兼発行　日本原子力研究所
印　　刷　㈱原子力資料サービス

JAERI-M 94-070

Radiation Protective Effects of Cysteamine and Glutathione on Four Nucleobases and Ascorbic Acid in Aqueous Solution

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(Received March 11, 1994)

This paper shows the radiation protective effects of cysteamine and glutathione (reduced form) on the π-systems of four nucleobases in aqueous solution. Thymine, cytosine, adenine, and guanine solutions containing cysteamine (or glutathione) in various concentrations were irradiated with gamma-rays from a 0.22 PBq Co-60 source. The residual concentration of each nucleobase after irradiation was measured by spectrophotometry and liquid chromathography. The π-systems of cytosine and adenine were protected and repaired by adding about a 3∿6 fold excess of cysteamine in relative to the cytosine and the adenine concentration. Each amino group, cytosine and adenine, plays an important role for the protection of their own π-system from the radiation damage. The π-system of thymine was protected from the radiation damage, but was not repaired by cysteamine. The protective effects of cysteamine on guanine were saturated with increasing the cysteamine concentration. Ascorbic acid solutions containing cysteamine were irradiated also. The radiation protective effect of cysteamine on ascorbic acid was small. A first approximation analysis was made on the radiation protective effects. The autooxidation of ascorbic acid in aqueous solution containing several kinds of antioxidants was measured and analyzed.

Keywords: Radiation, Protection, Repair, Scavenger, Thymine, Cytosine Adenine, Guanine, Ascorbic Acid, Cysteamine

JAERI-M 94-070

水溶液中の４つの核酸塩基とアスコルビン酸に対するシステアミンとグルタチオンの放射線防護効果

日本原子力研究所原子力総合研修センター
末武　雅晴

（1994年３月11日受理）

水溶液中の４つの核酸塩基とアスコルビン酸に対するシステアミンとグルタチオンの放射線防護特性を測定した。シトシンとアデニンのπ系は約３～６倍の濃度のシステアミンによって防護，修復される。シトシンとアデニンのアミノ基はそれぞれのπ系の放射線防護に重要な役割を果している。チミンのπ系はシステアミンによって防護されるが，修復はない。グアニンのπ系はシステアミン濃度をいくら増加しても飽和する放射線防護特性を示した。アスコルビン酸に対するシステアミンの放射線防護特性はグアニンと同様にシステアミン濃度を増加しても飽和し，その防護効果は小さい。システアミンと比較してグルタチオンの放射線防護効果は少なく，低線量照射ではグルタチオン濃度を増加すると，かえってその効果が少なくなる負の防護特性を示す。種々の抗酸化剤を含む水溶液中のアスコルビン酸の自動酸化特性を測定し，解析を試みてある。

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Abstract: This paper shows the radiation protective effects of cysteamine and glutathione (reduced form) on the π-systems of four nucleobases in aqueous solution. Thymine, cytosine, adenine, and guanine solutions containing cysteamine (or glutathione) in various concentrations were irradiated with gamma-rays from a 0.22 PBq Co-60 source. The residual concentration of each nucleobase after irradiation was measured by spectrophotometry and liquid chromatography. The π-systems of cytosine and adenine were protected and repaired by adding about a 3-6 fold excess of cysteamine in relative to the cytosine and the adenine concentration. Each amino group, cytosine and adenine, plays an important role for the protection of their own π-system from the radiation damage. The π-system of thymine was protected from the radiation damage, but was not repaired by cysteamine. The protective effects of cysteamine on guanine were saturated with increasing the cysteamine concentration. A first approximation analysis was made on the radiation protective effects of cysteamine and glutathione on four nucleobases.

抄録。 この論文では水溶液中のチミン、グアニン、シトシン、アデニンの４種の核酸塩基に対するシステアミンとグルタチオンの放射線防護特性が示されている。濃度の異なるシステアミン（又はグルタチオン）を含む一定濃度の核酸塩基を０．２２ＰＢｑの^{60}Ｃｏで照射して、その残留濃度を分光光度計と高速液体クロマトグラフで測定した。シトシンとアデニンのπ系は約３～６倍の濃度のシステアミンによつて、防護、修復され易い。シトシン、アデニンのアミノ基はそれぞれのπ系の放射線防護に重要な役割を果している。チミンのπ系はシステアミンによつて防護されるが、修復はない。グアニンに対するシステアミンの放射線防護効果はシステアミン濃度をいくら増加しても飽和する特性をもつている。これらの核酸塩基に対するシステアミンとグルタチオンの放射線防護特性を一次近次的に解析してある。

Keywords: Radiation protection, Repair, Radical scavenger, Thymine, Cytosine, Adenine, Guanine, Cysteamine, Glutathione(reduced form).

Introduction

To study the radiation protective effects of cysteamine and gultathione(reduced form), four nucleobases: thymine, cytosine, adenine and guanine were used as a target of irradiation. Cysteamine and glutathione are known for their radiation protective properties, as a radical scavenger of the water radical species or a chemical repair agent of the radical compounds[1][2][3][4].

Despite numerous investigations on the radiation protective effects of cysteamine (or glutathione) on the cellular systems, few quantitative explanations have been made until now[6][7][8][9]. The purpose of this report is to clarify quantitatively the radiation protective effects of cysteamine and glutathione on four nucleobases in aqueous solution.

The results are presented in this paper.

Experimental section.

All aqueous solutions were prepared with triply distilled water. Gamma-rays were provided by a 0.22 PBq Co-60 source. Chemicals used were obtained from Wako Junyaku Co.. Thymine, cytosine, adenine and guanine concentrations were determined by spectrophotometry and liquid chromathography. Millimollar solutions of each nucleobase containing cysteamine or glutathione in various concentrations were irradiated at three different dose levels: 1.1×10^2, 1.7×10^2 and 2.3×10^2 Gy. All measurements were made with a Shimazu UV-2100S spectrophotometer and a Shimazu LC-6A high performance liquid chromathography.

1. The π-system of thymine.

1.1 Results.

The fraction of residual to initial concentration of thymine, P, is shown in Fig.1.1 as a function of total dose. Curves obtained correspond to cysteamine concentrations added to thymine solution.

Fig.1.2 shows the changes in reciprocal fraction of damaged thymine,

1/(1-P), as a function of molar ratio x of cysteamine to thymine. Error bands at each measuring point are also shown together. The slope of a characteristic curve in the figure shows the rate constant ratio for water radical reaction with cysteamine and thymine, k, at a given dose, as will be discussed below.

1.2 Analysis and Discussion.

If the concentration of water radicals generated by γ-rays is [R], the relation between the water radical number and the damaged thymine concentration per unit time in the absence of cysteamine can be represented as follows.

$$-\frac{d[R]}{dt} = -n(0)\frac{d[T]}{dt} = n(0)k_t[T].[R]$$

where, k_t is the average (or macroscopic) rate constant of water radical reaction to break the π-system of thymine, [T] is the initial thymine concentration, and n(0) is the average number of water radicals to break the π-system of one thymine molecule in the absence of cysteamine. The damaged thymine concentration in the absence of cysteamine, $(\Delta T_P)_0$, by the primary reaction of water radicals can be written as follows,

$$(\Delta T_P)_0 \sim [R]/n(0),$$

or
$$[T]/(\Delta T_P)_0 = 1/(1-P)_0 \sim n(0)[T]/[R]. \qquad (1.1)$$

The n(0) is given as below, approximately.

$$n(0) = \frac{G(\text{water radicals})}{G(\text{-thymine})} = \frac{6.05}{2.6} = 2.4,$$

where, the G(water radicals)= G(OH) + $G(e^-_{aq})$ + G(H); G(OH)=2.75, $G(e^-_{aq})$=2.65, and G(H)=0.65, is used. The primary water radical species, e^-_{aq} and H form HO_2 or O^-_2 in the presence of oxygen in water. The OH and HO_2 react and break the π- system of thymine.

The π- system of thymine is changed to the satulated σ-system of thymine through two step process of the water radicals, at least. An unstable thymine radical is formed at the first step by the additive reaction of a water radical, OH to thymine.

$$T + OH \rightarrow T\cdot\text{-}OH \qquad (1.2)$$

The thymine radical reacts with another water radical species, such as $H\cdot$ or $HO\cdot_2$, and forms a hydroperoxid of thymine which is thought to be considerably stable.

$$T\cdot\text{-}OH + HO_2 \text{ (or } H\cdot) \rightarrow T < \begin{matrix} OOH(\text{or } H) \\ OH \end{matrix}$$

Table 1.1 lists the reaction indices of thymine at each atom position: the superdelocalizabilities, S_r.[5] The π-system of thymine is broken at C_6 and/or C_5 by the water radical reactions, either phil-electronic or phil-nucleic, anyway. Table 1.2 lists the reaction indices of thymine radical, which is formed with the additive reaction of OH radical to C_6 of thymine. The thymine radical is considerably unstable at it's C_5 position and a short lived molecule in aqueous solution. The π-system of one thymine molecule is broken at least by two water radical species in the absence of cysteamine, approximately.

If cysteamine is added to thymine solution, a competitive reaction of water radicals to thymine and cysteamine takes place in the solution. If the reaction of water radicals takes place independently of thymine and cysteamine, the reaction rate of water radicals can be written as follows,

$$-\frac{d[R]}{dt} = -\left(\frac{n(0)d[T]}{dt} + \frac{d[SH]}{dt}\right) = (n(0)k_t[T] + k_{SH}[SH])\cdot[R] \ .$$

where, k_{SH} is the average rate constant of water radical reaction with cysteamine, and [SH] is the initial cysteamine concentration. Then, the primary reaction probability of water radicals with thymine, $(W'_t)_x$ can be represented as follows,

$$(W'_t)_x = \frac{d[T]}{dt} \Big/ \frac{d[R]}{dt} = \frac{n(0)k_t[T]}{n(0)k_t[T] + k_{SH}[SH]}$$

The damaged thymine concentration by the primary reaction, $\Delta T_P'$ can be written as follows,

$$\Delta T_P' = \frac{[R]}{n(0)} \frac{n(0)\,k_t[T]}{n(0)k_t[T] + k_{SH}[SH]} \ ,$$

$$\sim \frac{[R]}{2} \frac{2k_t[T]}{2k_t[T] + k_{SH}[SH]} \ .$$

where, $n(0)\sim 2$ is assumed instead of $n(0)=2.4$. Then,

$$[T]/\ \Delta T_P' = (1/(1-P))_x \sim \frac{2[T]}{[R]}\ (1 + \frac{1}{2}\,kx).$$

where, $k = k_{SH}/k_t$, and $x = [SH]/[T]$.

With increasing cysteamine concentration, however, a thymine radical formed by reaction (1.2) begins to interact with cysteamine. The following reaction takes place in a dense cysteamine solution.

$$T^{\cdot}\text{-}OH + RSH \rightarrow T<^{H}_{OH} + RS^{\cdot} \qquad (1.3)$$

The thymine radical extracts another hydrogen from cysteamine, and forms a hydroxythymine. In the dense cysteamine solution, one thymine molecule forms one hydroxythymine with one water radical species.

If n(x) is the average number of water radicals to break the π-system of one thymine molecule in the solution of molar ratio x of cysteamine to thymine, the following relations hold.

$$-\frac{d[R]}{dt} = -\left(\frac{n(x)d[T]}{dt} + \frac{d[SH]}{dt}\right) = (n(x)k_t[T] + k_{SH}[SH])\cdot[R].$$

The reaction probability of water radicals with thymine in dense cysteamine solution, W_t can be written as follows,

$$(W_t)_x = \frac{d[T]}{dt} \Big/ \frac{d[R]}{dt} = \frac{n(x)k_t[T]}{n(x)k_t[T] + k_{SH}[SH]} .$$

The n(x) depends on the interaction of the thymine radicals and cysteamine, and so must be a function of the molar ratio x. If one water radical species reacts with one thymine molecule and breaks the π-system of thymine, the damaged thymine concentration by the primary reaction of water radicals, ΔT_P can be approximated as follows,

$$\Delta T_P = \frac{[R]}{n(x)} \frac{n(x)\ k_t[T]}{n(x)k_t[T] + k_{SH}[SH]} .$$

$$\sim [R] \frac{k_t[T]}{k_t[T] + k_{SH}[SH]} ,$$

where, n(x)=1 is assumed in the dense cysteamine solution. Then,

$$[T]/\Delta T_P = (1/(1-P))_x \sim \frac{[T]}{[R]}(1 + kx). \qquad (1.4)$$

As shown in the Fig.1.2, the rate constant ratio of water radical reaction with cysteamine and thymine, k were obtained in 2.0～2.1 within the region of $1<x<4$ at each dose level.

The dose reduction factor, DRF of radiation protective agent in the

radiation biology is defined as the ratio of 37% dose with and without the protective agent.

$$DRF \equiv \frac{D_{37}(\text{with protective agent})}{D_{37}(\text{without protective agent})} \tag{1.5}$$

It may be permitted to define a radiation protection factor, F in a simple biochemical system, as follows.

$$F \equiv \frac{\Delta T(\text{without protective agent})}{\Delta T(\text{with protective agent})}, \tag{1.6}$$

where, ΔT is the damaged thymine concentration with or without the radiation protective agent at a given dose. By using the reciprocal fraction of damaged thymine concentration, 1/(1-P), the protection factor F at molar ratio x can be represented as follows,

$$F \equiv \frac{(1/(1-P))_x}{(1/(1-P))_0} \tag{1.7}$$

The damaged thymine concentration at low dose ($1\sim3\times10^2$ Gy) increases almost linearly with dose, as shown in the Fig.1.1. Therefore, Eq(1.6) is equivalent to Eq(1.5), approximately. The Eq(1.6) is more suitable than Eq(1.5) for the evaluation of dose dependency related to the radiation protective effects.

Then, the radiation protection factor, F of cysteamine for thymine can be written as follows, from Eq(1.7).

$$F \equiv \frac{(1/(1-P))_x}{(1/(1-P))_0} \sim \frac{n(x)}{n(0)}\left(1 + \frac{k_{SH}}{n(x)k_t}\,x\right) \tag{1.8}$$

where,

$$n(x) = 2 \rightarrow 1, \quad 0<x<1$$
$$= 1, \quad 1<x<4.$$

In Fig.1.3, the experimental curve is almost a straight line in the region B of $1<x<4$. The curve of the protection factor F begins from the point of 1 at $x=0$, and shifts to the straight line, which has the contact point of about 1/2 to the ordinate, with increasing x. This may indicate that the π-system of one thymine molecule is broken by about two water radical species in the absence of cysteamine, but by one water radical species and one hydrogen from cysteamine as cysteamine concentration increases.

As shown in the figure, the n(x) in a dilute cysteamine solution of region A (0<x<1) can be approximated as follows,

$$n(x) \sim n(0)(1 + k_n x).$$

Then,
$$F = \frac{n(x)}{n(0)} \left(1 + \frac{k_{SH}}{n(x)k_t} x \right) \sim 1 + \left(k_n + \frac{k}{n(0)} \right) x ,$$
$$= 1 + k'x . \qquad (1.9)$$

where, $k' = k_n + k/n(0)$, in dilute cysteamine solution.

By using the experimental values,

$$F \sim 1 + 0.5x ,$$

and,
$$n(x) = n(0)(1 - 0.8x).$$

The value of k_n is negative. This means that the number of water radicals to break the π-system of thymine decreases rapidly with increasing cysteamine concentration, as discussed before.

In region C(4<x<6) of the figure, the radiation protection factor F is saturated slightly with molar ratio x. The secondary reaction of cysteamine radicals with thymine seems to take place in a dense cysteamine solution. The cysteamine radical produced by the primary reaction of water radicals combines easily with the dissolved oxygen O_2 in water and forms a compound radical $RS \cdot O_2$. The compound radical reacts to hydrogenperoxide H_2O_2 which is accumulated in irradiated solution, and generates a superoxide radical $HO \cdot_2$. The superoxide radical may react with thymine again[3].

If the damaged thymine by the secondary reaction of the superoxide radical, ΔT_S, is small and constant, the net amount of the damaged thymine by the indirect effects, ΔT_{ID}, can be written as follows.

$$\Delta T_{ID} = \Delta T_P + \Delta T_S$$

or
$$\frac{1}{\Delta T_{ID}} \sim \frac{1}{\Delta T_P}\left(1 - \frac{\Delta T_S}{\Delta T_P}\right).$$

where, $\Delta T_S / \Delta T_P < 1$ is assumed.

The above equation can be approximated as follows.

$$\frac{[T]}{\Delta T_{ID}} \sim \frac{[T]}{[R]}\left(1 + kx - \frac{\Delta T_S}{[R]} k^2x^2 \right) .$$

or, from Eq(1.8),

$$F \sim \frac{1}{2} \left(1 + kx - \frac{\Delta T_S}{[R]} k^2x^2 \right) , \qquad (1.10)$$

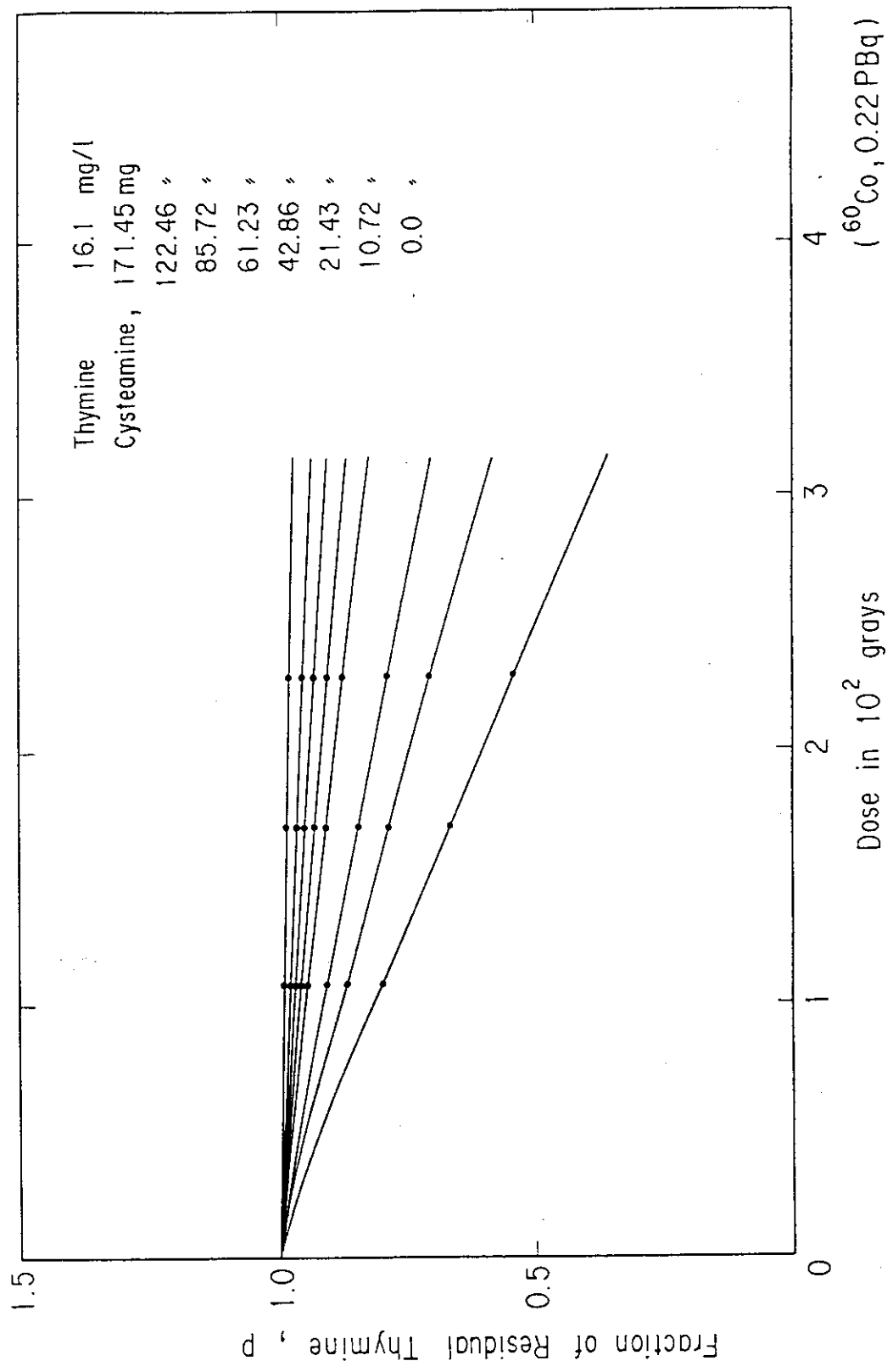


Fig. 1.1 Fraction of residual to initial concentration of thymine in aqueous solutions containing cysteamine, 24hr post irradiation.

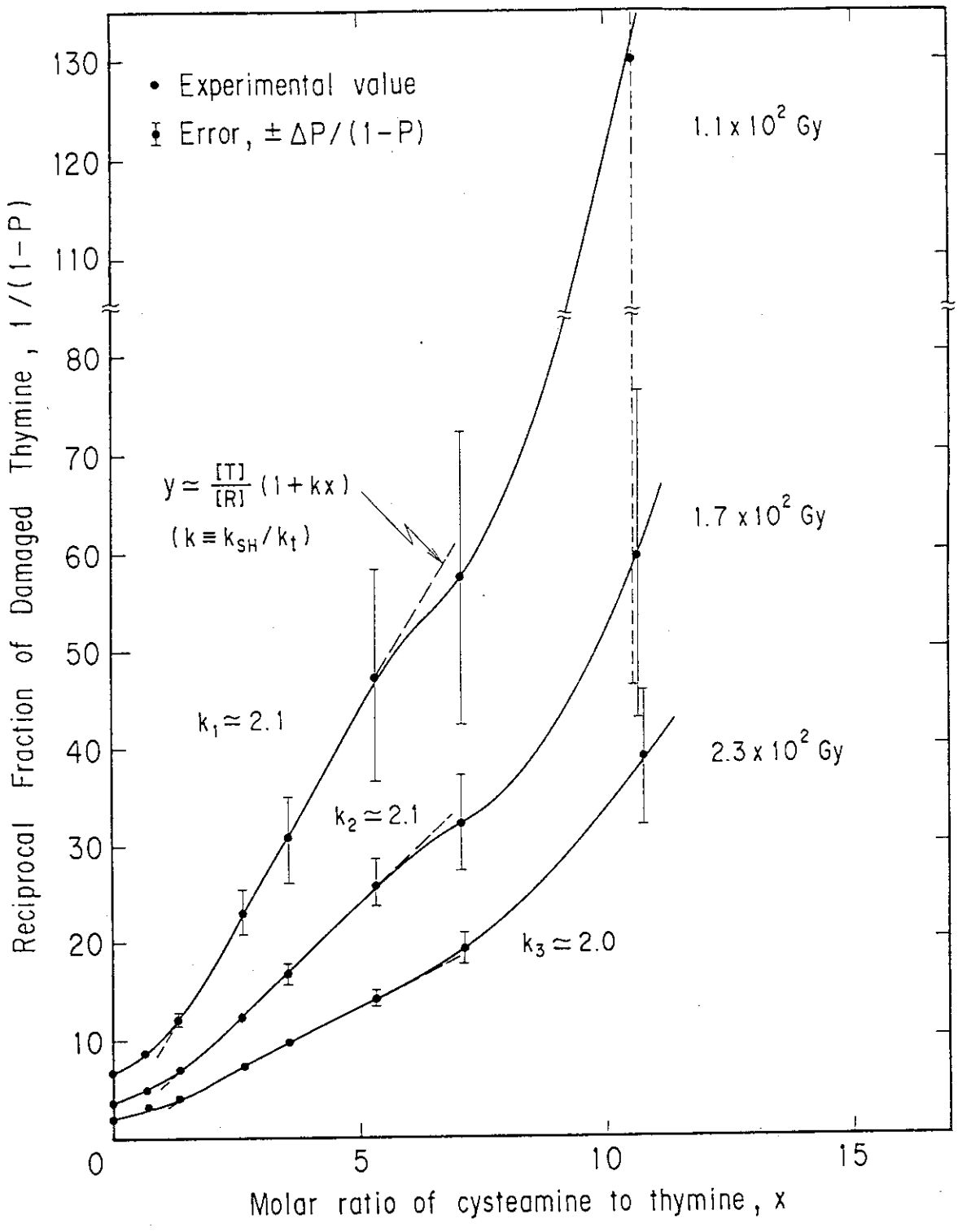


Fig. 1.2 Reciprocal fraction of damaged thymine, as a function of molar ratio of cysteamine to thymine.

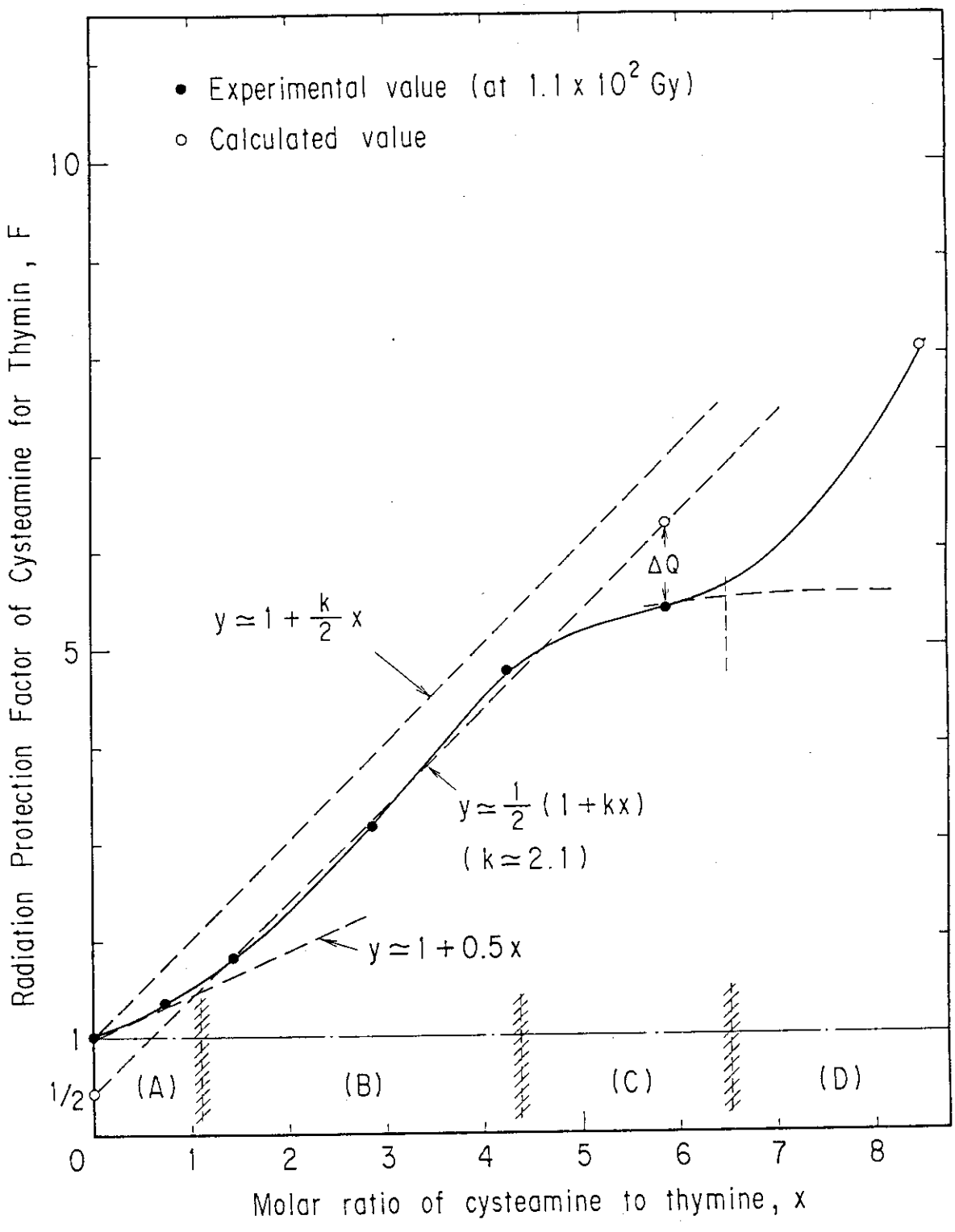
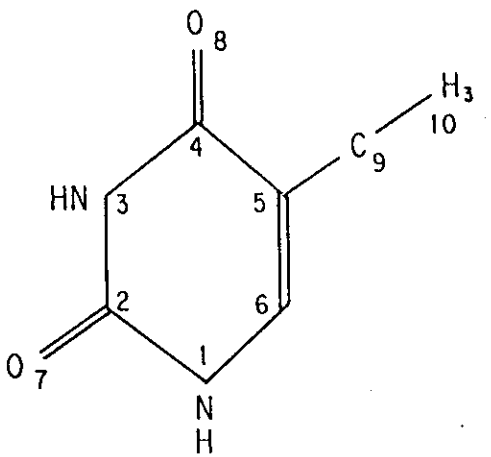


Fig. 1.3 The calculated radiation protection factor of cysteamine for thymine in aqueous solution.

Table 1.1 The reaction indices of thymine, S_r*.

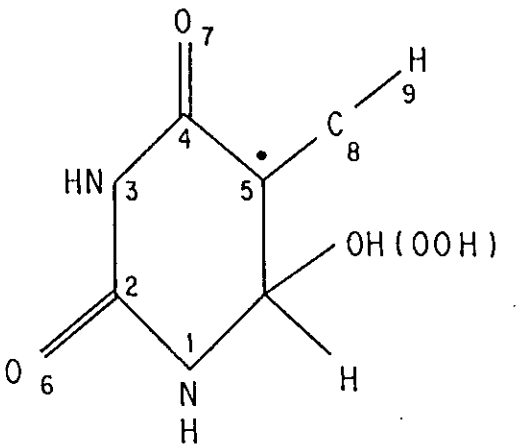


Superdelocalizabilities :

	1	2	3	4	5	6	7	8	9	10
$S_r^{(E)}$	0.5047	0.4445	0.9862	0.4100	1.1564	0.5956	0.8641	0.8932	0.4850	0.4814
$S_r^{(N)}$	0.5146	1.1268	0.4963	1.1398	0.6616	1.3047	0.2427	0.2580	0.3222	0.4097
$S_r^{(R)}$	0.5096	0.7856	0.7412	0.7749	0.9090	0.9501	0.5534	0.5756	0.4036	0.4455

* HMO calculations were done using parameters suggested by T. Yonezawa , et al. (5)

Table 1.2 The reaction indices of thymine radical, S_r*.



Superdelocalizabilities :

	1	2	3	4	5	6	7	8	9
$S_r^{(E)}$	1.4047	0.3171	1.2148	0.5806	1.6846	0.8272	0.9625	0.4749	0.5057
$S_r^{(N)}$	0.3591	1.1538	0.5599	1.1020	2.0796	0.2652	0.3731	0.3387	0.5360
$S_r^{(R)}$	0.8819	0.7355	0.8874	0.8413	1.8821	0.5462	0.6678	0.4068	0.5208

* HMO calculations were made on thymine radical π-anion.
One electron was extracted from HOMO of the thymine radical π-anion.

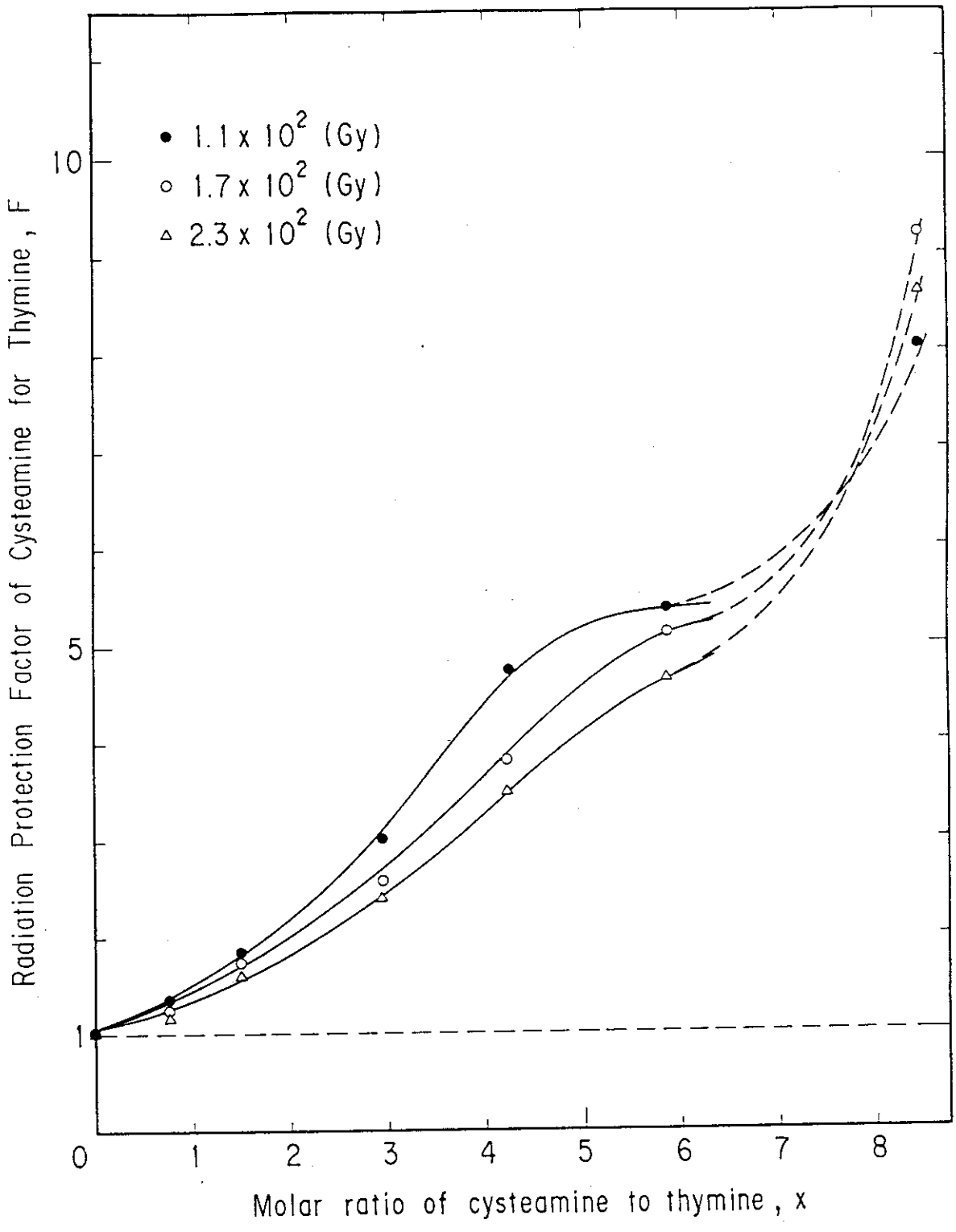


Fig. 1.4 Radiation protection factor of cysteamine for thymine in aqueous solution as a function of molar ratio of cysteamine to thymine.

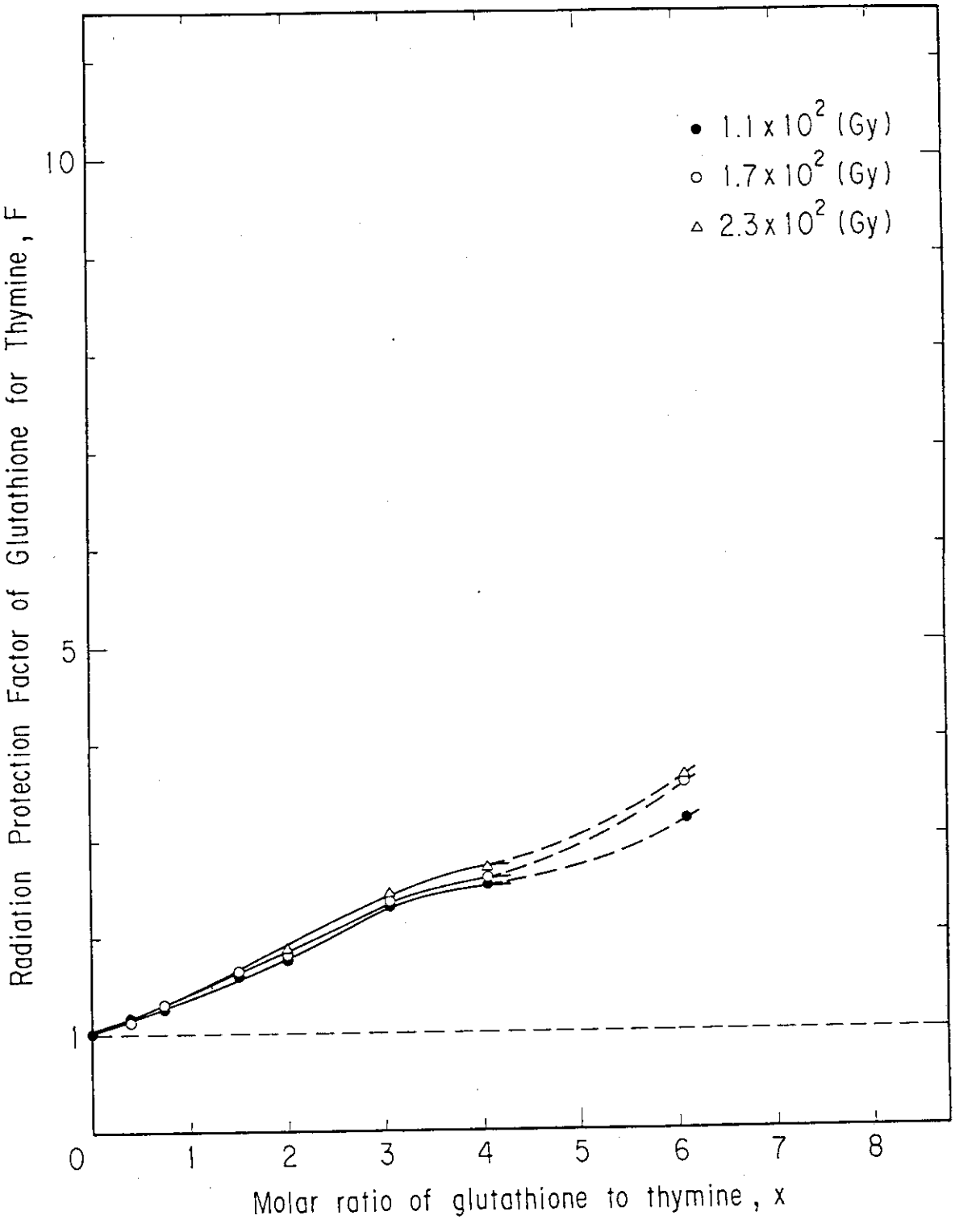


Fig. 1.5 Radiation protection factor of glutathione for thymine in aqueous solution as a function of molar ratio of glutathione to thymine.

where, $\Delta T_S < [R]$ is assumed.

The third term in the parenthes of Eq(1.10) represents the secondary reaction with the superoxide radicals. The difference, ΔQ, between the experimental value and the extrapolated value of the straight line ($1<x<4$) in Fig.1.3 is represented as follows.

$$\Delta Q \sim \frac{1}{2[R]} \Delta T_S \cdot k^2 x^2 ,$$

or,

$$\frac{\Delta T_S}{[R]} \sim \frac{2\Delta Q}{k^2 x^2} = 1.3 \times 10^{-2}$$

The secondary reaction appears in the cases of low dose at 1.1×10^2 and 1.7×10^2 Gy. This means that the secondary reaction becomes prominent only if the dissolved oxygen concentration in water is comparable to water radical concentration generated by irradiation. The secondary reaction is estimated to have contributed about 2.6% of the damaged thymine concentration ($\sim[R]/2$) in the absence of cysteamine.

In region D ($x>6$) of the Fig.1.3, the curve increases rapidly with x, again. It can be considered that the enol-form of thymine, or some other compounds of thymine radical and cysteamine, which have much stronger absorbance than the original thymine, are formed by irradiation in dense cysteamine solution.[10][11]

Fig.1.4 shows the radiation protection factor F of cysteamine for thymine. As shown in the figure, the protection factor F reaches 4～5 at molar ratio x=4. This means that the damaged thymine concentration at the molar ratio x=4 is decreased to one fourth or one fifth of that in the absence of cysteamine. Fig.1.5 shows the radiation protection factor of glutathione for thymine. Glutathione is not so good a protective agent for thymine as cysteamine .

1.3 Conclusion.

The above analysis result leads to the following conclusion;

(1) The radiation protection factor of cysteamine for thymine is not increased so much in a dilute cysteamine solution of molar ratio $x<1$. However, the radiation protection factor reaches about 4～5 in the

solution with molar ratio x=4, at low dose(<$2x10^2$ in grays). The damaged thymine concentration at the x=4 is reduced to one fourth or one fifth of that in which no cysteamine is added.

(2) One thymine molecule needs at least two water radicals to form one hydroperoxide of thymine in the absence of cysteamine. Intermediate thymine radical interacts with cysteamine, and extracts one hydrogen from cysteamine. As cysteamine concentration is increased, one thymine molecule forms one saturated hydroxythymine with one water radical and one hydrogen from cysteamine.

(3) The protection factor is saturated with increasing cysteamine concentration over x>5 at a low dose. In the presence of dissolved oxygen in water, the secondary reaction of superoxide radical with thymine takes place in the dense cysteamine solution.

Cysteamine cannot repair chemically the π-system of thymine on the way of breaking. Cysteamine accellerates the transformation of thymine from the π-system to the σ-system in dilute cysteamine solution. A dense cysteamine solution of molar ratio x$>$1 is needed to protect thymine from the radiation damage in aqueous solution.

2. The π-system of cytosine.

2.1 Results.

The fraction of residual to initial concentration of cytosine, P is shown in Fig.2.1 as a function of total dose. Each curves in the figure correspond to cysteamine concentrations added to cytosine solution. Some of the P values in dense cysteamine solutions exceed unity. In Fig.2.2 curves show the changes in reciprocal fraction of damaged cytosine, 1/(1-P) as a function of molar ratio x of cysteamine to cytosine. The characteristic curves in the figure indicate that cysteamine repair chemically the π-system of cytosine on the way of breaking, as will be discussed below.

2.2 Analysis and Discussion.

As in the case of thymine, the primary reaction probability of water radicals to cytosine, W_C, in the solution of molar ratio x of cysteamine to cytosine, can be written as follows.

$$(W_C)_x = \frac{n(x)k_C[C]}{n(x)k_C[C] + k_{SH}[SH]}$$

where, k_C is the average rate constant for water radical reactions to break the π-system of cytosine, [C] is the initial cytosine concentration, k_{SH} is the rate constant of water radical reaction with cysteamine, [SH] is the initial cysteamine concentration, and n(x) is the average number of water radicals to break the π-system of cytosine in the solution of molar ratio x of cysteamine to cytosine. Then, the damaged cytosine concentration by the primary reaction of water radicals, ΔC_P, can be written as follows.

$$\Delta C_P \sim \frac{[R]}{n(x)} \frac{n(x)k_C[C]}{n(x)k_C[C] + k_{SH}[SH]}$$

If the repair reaction of cysteamine for the damaged cytosine on the way of breaking takes place in the solution, the repaired cytosine concentration, ΔC_R, may be written as follows.

$$\Delta C_R = k_R[SH]\Delta C_P \sim \frac{[R]}{n(x)} \frac{n(x)\cdot k_R \cdot k_C[C][SH]}{n(x)k_C[C] + k_{SH}[SH]}$$

where, [SH]>[R] is assumed, and k_R is the repair probability of cysteamine for the damaged cytosine on the way of breaking. Then, the net concentration of the damaged cytosine by the indirect effects, ΔC_{ID}, will be represented as follows,

$$\Delta C_{ID} = (\Delta C_P - \Delta C_R) \sim \frac{[R]}{n(x)} \frac{n(x)(k_C[C] - k_R \cdot k_C[C][SH])}{n(x)k_C[C] + k_{SH}[SH]},$$

or,

$$\frac{1}{\Delta C_{ID}} \sim \frac{n(x)}{[R]} \frac{k_C[C] + k_{SH}[SH]/n(x)}{k_C[C] - k_R \cdot k_C[C][SH]} = \frac{n(x)}{[R]} \frac{(1 + kx/n(x))}{(1 - k_R[SH])}$$

$$= \frac{n(x)}{[R]} \frac{(1 + kx/n(x))}{(1 - k_R[C]x)} . \qquad (2.1)$$

where, $k = k_{SH}/k_C$, $x = [SH]/[C]$.

The radiation protection factor F, defined in Eq(1.10) of thymine case will be given as follows,

$$F = \frac{n(x)}{n(0)} \quad \frac{1 + kx/n(x)}{1 - k_R[C]x}$$

$$\sim \frac{1 + k'x}{1 - k_R[C]x} \quad , \tag{2.2}$$

where, $n(x) = n(0)(1 + k_n x)$ in dilute cysteamine solution, and

$$\frac{n(x)}{n(0)} (1 + kx/n(x)) \sim 1 + k'x, \tag{2.3}$$

$$k' = k_n + k/n(0),$$

are assumed, as in the case of thymine. The n(x) is considered to be constant in a dense cysteamine solutions.

Fig.2.3 shows the calculated radiation protection factor F of cysteamine for cytosine at 1.1×10^2 Gy. As shown in the figure, the variation of F can be approximated as follows.

In a dilute cysteamine solution with $0<x<1$,

$$F = y \sim 1 + 0.75x ,$$

$$k' \sim 0.75 .$$

The values of k and k_n in Eq (2.3) could not be obtained separately. The k' value is small. This means that the radiation protective effect of cysteamine on cytosine is not so large in a dilute cysteamine solution with molar ratio $x<1$. As in the case of thymine, an intermediate cytosine radical produced by the primary reaction of water radicals may interact with cysteamine.

In a dense cysteamine solution with $x>2$,

$$F = y \sim \frac{1 + 0.44x}{1 - 0.311x}$$

then, $k' \sim 0.44$,

$$k_R[C] \sim 0.311.$$

Eq(2.2) indicates that if the molar ratio x is increased to $1/k_R[C]$, the F value becomes infinite. This means that the π-system of cytosine in a dense cysteamine solution is completely protected by cysteamine during irradiation.

$$(x)_{y=\infty} = 1 / k_R[C] = 3.21$$

The π-system of one cytosine molecule can be protected and repaired chemically by about three cysteamine molecules in aqueous solution from the radiation damage .

Table 2.1 lists the reaction indices of cytosine molecule: the superdelocalizabilities, $S_r^{(R)}$. The highest value of the $S_r^{(R)}$ appears at N_8 of cytosine. It means that a water radical extracts one electron from the N_8 position. In the presence of cysteamine, one electron will be supplied from the cysteamine to the N_8 position of cytosine radical which is formed by the extractive reaction of water radicals.[5]

The chemical repair process of cysteamine for cytosine can be represented as follows.

$$\underset{}{\overset{NH_2}{C}} + O\cdot H \xrightarrow[(-H_2O)]{\text{(extraction)}} \overset{\cdot NH}{C} + RSH \xrightarrow{\text{(repair)}} \overset{NH_2}{C} + RS\cdot \qquad (2.4)$$

Fig.2.4 shows the radiation protection factor F of cysteamine for cytosine. The radiation protection factor depends sligtly on dose.

As shown in Fig.2.1, the fraction of residual cytosine concentration, P exceeds unity in a dense cysteamine solutions ($x>4$). The experimental results show that some other compounds, which have a higher absorbance than initial cytosine, are formed.

$$\underset{}{\overset{NH_2}{C}} + O\cdot H \xrightarrow[(-H_2O)]{\text{(extraction)}} \overset{\cdot NH}{C} + RSH \xrightarrow{\text{(rearrangement)}} {}^{H}\overset{NH}{C} + RS\cdot \qquad (2.5)$$

The rearrangement, from amino form to imino form of cytosine, takes place in the dense cysteamine solution. Table 2.2 lists the reaction indices of the imino form of cytosine radical. The highest value of the $S_r^{(R)}$ appears still at N_8 of the cytosine radical. The cytosine radical is considerably stable. The protonation at N_8 position of cytosine radical may take place in dense cysteamine solution.[12][13][14] However, the compounds of cysteamine and cytosine radicals could not be separated in liquid chromathography.

Fig 2.5 shows the radiation protection factor F of glutathione for cytosine. Glutathine protects and repairs cytosine from radiation damage in a solution with molar ratio $x<7$. In a dense glutathione solution with molar ratio $x>7$, however, the protection factor is decreased slightly with increasing x. The secondary reaction of superoxide radicals produced by the reaction of glutathione radical

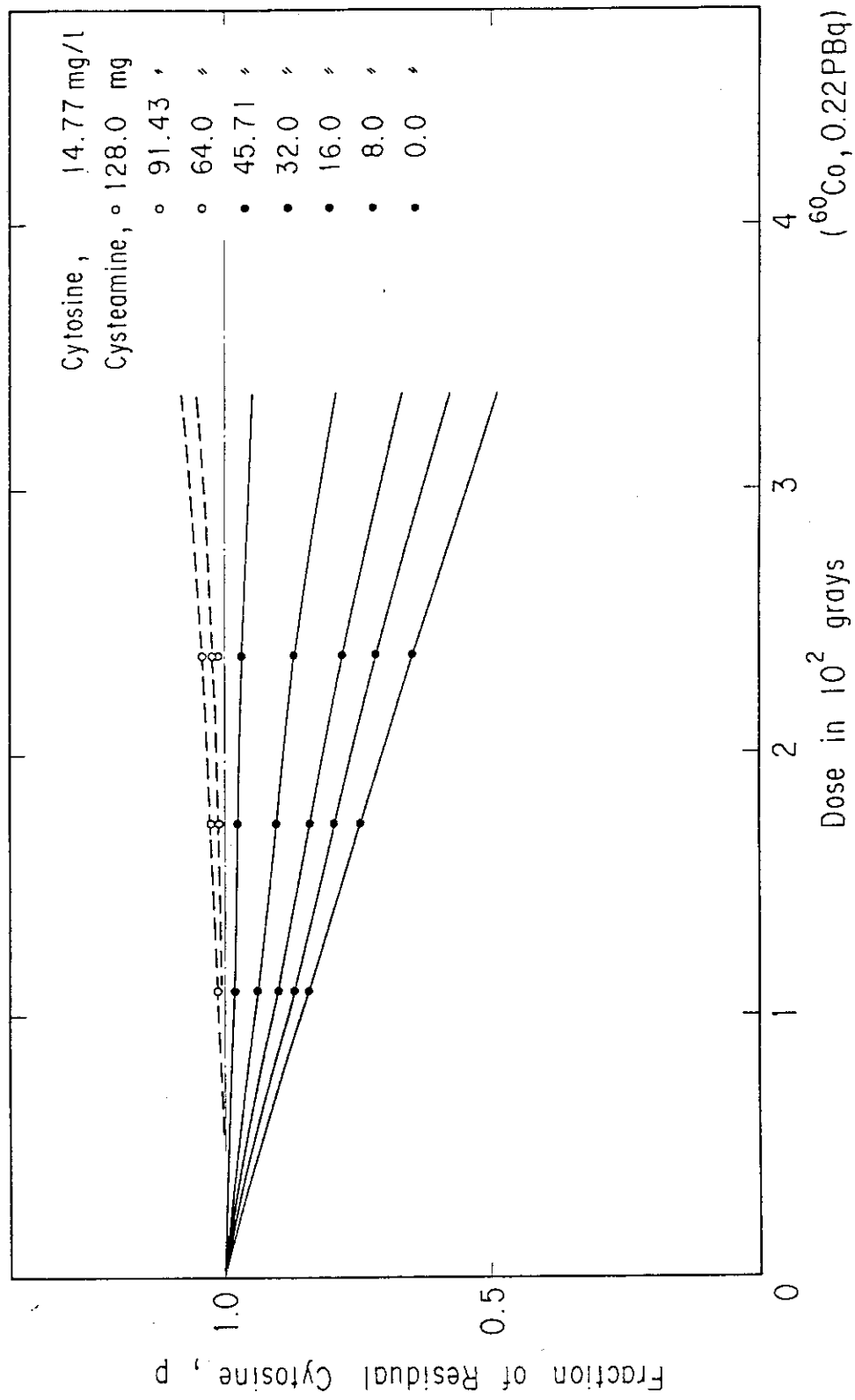


Fig. 2.1 Fraction of residual to initial concentration of cytosine in aqueous solutions containing cysteamine, 24hr post irradiation.

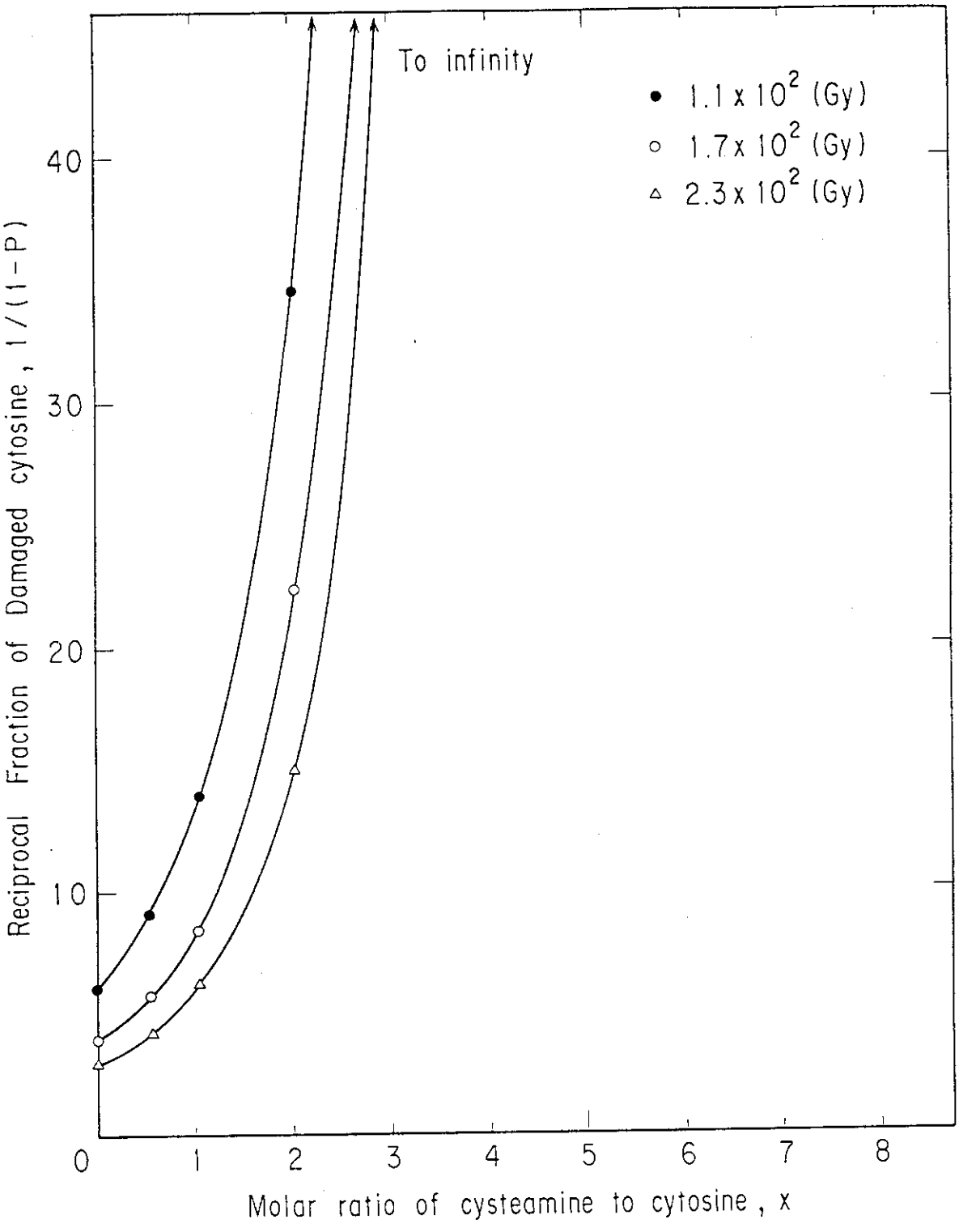


Fig. 2.2 Reciprocal fraction of damaged cytosine, as a function of molar ratio of cysteamine to cytosine.

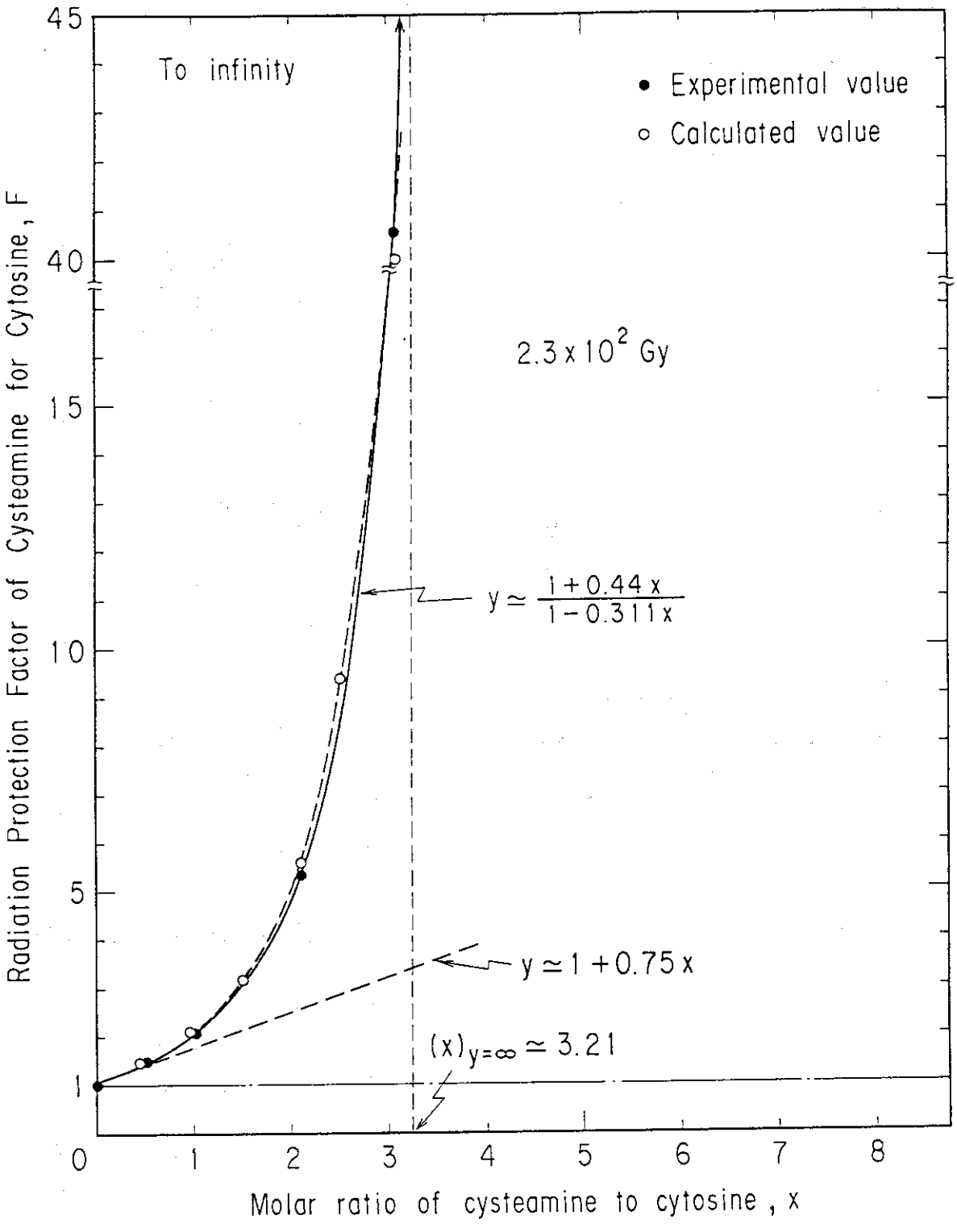
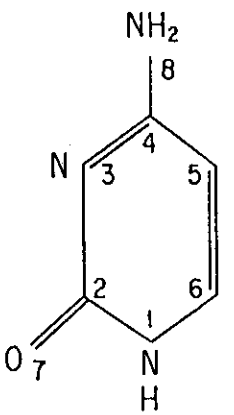


Fig. 2.3 The calculated radiation protection factor of cysteamine for cytosine in aqueous solution.

Table 2.1 The reaction indices of cytosine, S_r*.



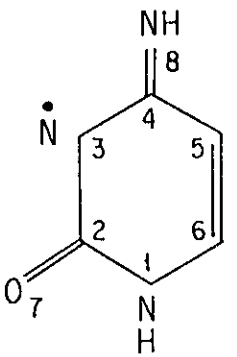
(lactam)

Superdelocalizabilities :

	1	2	3	4	5	6	7	8
$S_r^{(E)}$	0.8664	0.7241	0.9490	0.7108	1.1951	0.8339	0.5051	4.0175
$S_r^{(N)}$	0.6459	1.1083	0.7954	1.1710	0.3772	1.1029	0.6951	0.02837
$S_r^{(R)}$	0.7561	0.9162	0.8722	0.9409	0.7861	0.9684	0.6001	2.0229

* HMO calculations were done using parameters suggested by T. Yonezawa, et al. (5).

Table 2.2 The reaction indices of cytosine radical, S_r*.



Superdelocalizabilities :

	1	2	3	4	5	6	7	8
$S_r^{(E)}$	0.8071	0.6212	0.8113	0.7578	0.9600	0.5462	0.4995	1.6256
$S_r^{(N)}$	0.8128	1.1000	0.7689	0.9601	0.5557	1.4322	0.6898	1.0848
$S_r^{(R)}$	0.8099	0.8606	0.7901	0.8589	0.7578	0.9892	0.5946	1.3552

* HMO calculations were made on cytosine (imino form) π-anion.
One electron was extracted from HOMO of the cytosine π-anion.

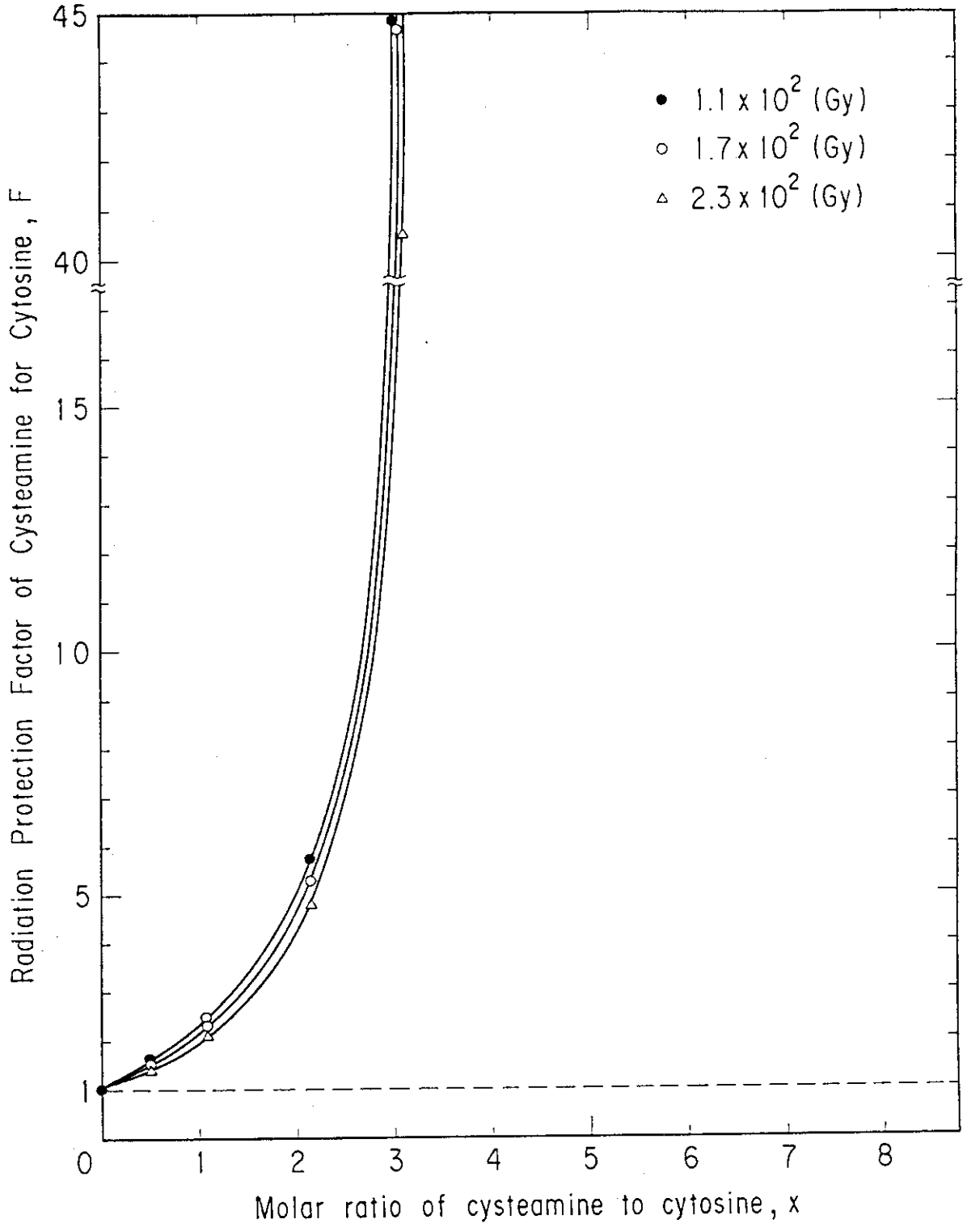


Fig. 2.4 Radiation protection factor of cysteamine for cytosine in aqueous solution as a function of molar ratio of cysteamine to cytosine.

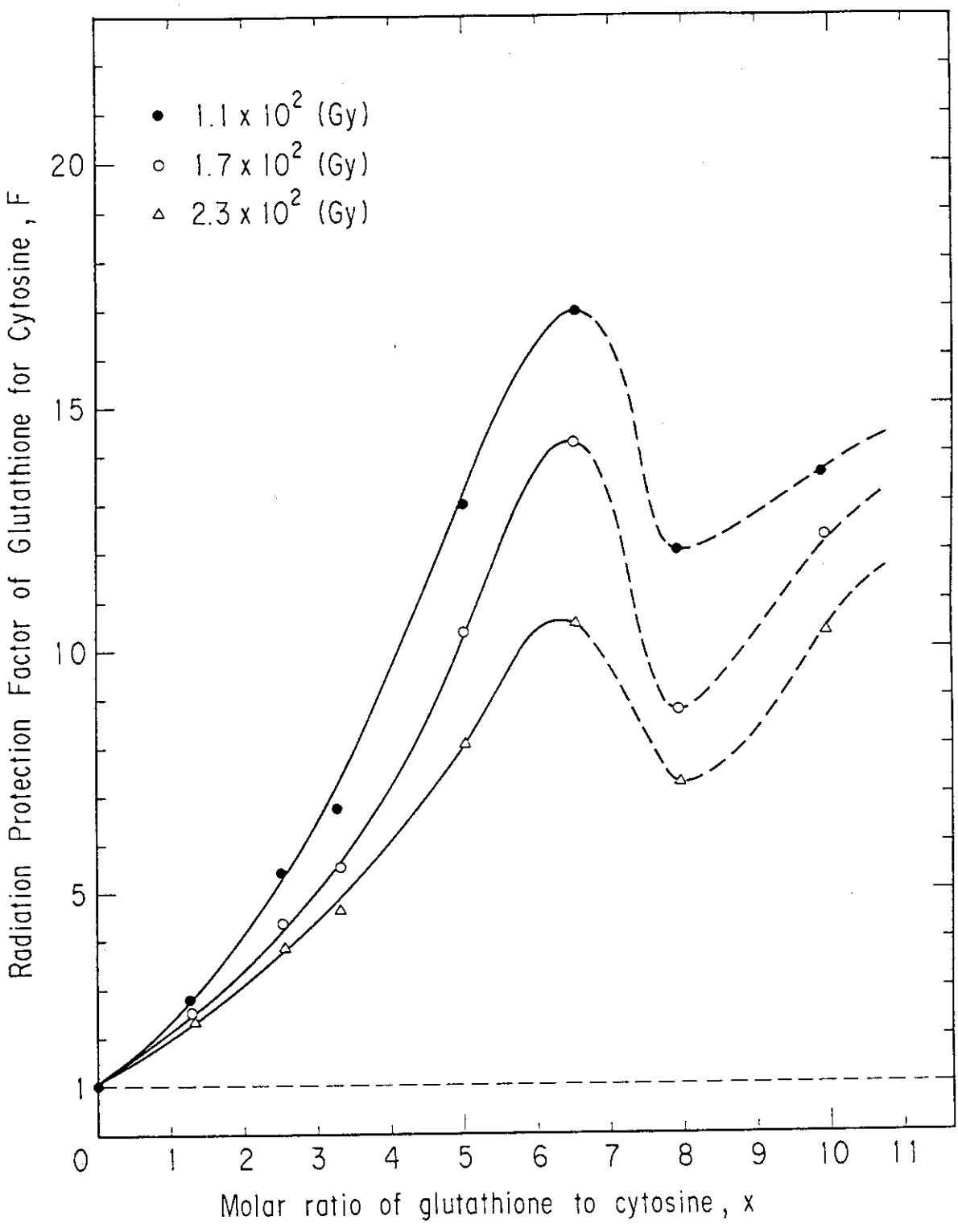


Fig. 2.5 Radiation protection factor of glutathione for cytosine in aqueous solution as a function of molar ratio of glutathione to cytosine.

with hydrogenperoxid may take place in the dense glutathione solution.

2.3 Conclusion

The above discussion leads to the following conclusion;

(1)The π-system of one cytosine molecule is protected and repaired chemically from the radiation damage by about three cysteamine molecules.

(2)The amino group of cytosine at N_8 position is easy to release one electron. A water radical extracts easily one electron from the amino group in the absence of cysteamine.

(3)In the presence of cysteamine, the amino radical formed at the N_8 position is repaired chemically by the supply of one electron from cysteamine. Although some kinds of compounds, which have much higher absorbance than the original cytosine, are formed in dense cysteamine solution, the π-system of cytosine is protected by cysteamine during irradiation.

The amino group of cytosine is electron rich. The water radicals extract easily one electron from the amino group. Intermediate cytosine radical interacts with cysteamine. The intermediate cytosine radical is repaired chemically by cysteamine. The amino group at N_8 position of cytosine protects the π-system of cytosine itself from the radiation damage in the presence of cysteamine.

3. The π-system of adenine.

3.1 Results.

The fraction of residual to initial concentration of adenine, P is shown in Fig.3.1 as a function of total dose. Each curve in the figure corresponds to different cysteamine concentrations added to adenine solution. As in the case of cytosine, some of the P values in dense cysteamine solutions exceed unity. Fig.3.2 shows the changes in reciprocal fraction of damaged adenine concentration, 1/(1-P) as a function of molar ratio x of cysteamine to adenine. The characteristic

curves in the figure indicate that cysteamine repairs chemically the π-system of adenine on the way of breaking, as in the case of cytosine.

3.2 Analysis and Discussion.

As in the case of cytosine, the net concentration of damaged adenine through the indirect effects, ΔA_{ID}, satisfies the following relationship.

$$\frac{1}{\Delta A_{ID}} \sim \frac{n(x)}{[R]} \frac{k_A[A] + k_{SH}[SH]/n(x)}{k_A[A]-k_R \cdot k_A[A][SH]} = \frac{n(x)}{[R]} \frac{(1 + kx/n(x))}{(1 - k_R[SH])}$$

$$= \frac{n(x)}{[R]} \frac{(1 + kx/n(x))}{(1 - k_R[A] \cdot x)} , \qquad (3.1)$$

where, $k = k_{SH}/k_A$, $x = [SH]/[A]$.

The k_A is the average rate constant for water radical reaction to break the π-system of adenine, [A] is the initial adenine concentration, [SH] is the initial cysteamine concentration. n(x) is the average number of water radicals to break the π-system of adenine in a solution with molar ratio x of cysteamine to adenine.

Then, the radiation protection factor F of cysteamine for adenine can be written as follows.

$$F = \frac{n(x)}{n(0)} \frac{1 + kx/n(x)}{1 - k_R[A] \cdot x} .$$

And,

$$\sim \frac{1 + k'x}{1 - k_R[A] \cdot x} . \qquad (3.2)$$

where, $n(x) = n(0)(1 + k_n x)$ in dilute cysteamine solution, and

$$\frac{n(x)}{n(0)} (1 + kx/n(x)) \sim 1 + k'x ,$$

$$k' = k_n + k/n(0) ,$$

are assumed, as in the case of cytosine.

Fig.3.3 shows the calculated radiation protection factor F of cysteamine for adenine at a dose of 2.3×10^2 Gy. As shown in the figure, the variation of F can be approximated as follows.

$$F = y \sim \frac{1 + 0.33x}{1 - 0.17x}$$

and hence,

$k' \sim 0.33,$

$k_R[A] \sim 0.17.$

Eq(3.2) indicates that if the molar ratio x of cysteamine to adenine is increased to $1/k_R[A]$, the F value becomes infinite. This means that the original π-system of adenine is completely protected by cysteamine from the radiation damage in dense cysteamine solutions with molar ratio x near $1/k_R[A]$.

$$(x)_{y=\infty} = 1 / k_R[A] = 5.88 \tag{3.3}$$

The damaged π-system of one adenine molecule is protected and repaired chemically by about six cysteamine molecules at 2.3×10^2 Gy.

Table 3.1 shows the reaction indices of adenine molecule.[5] The highest value of the superdelocalizabilities, $S_r^{(R)}$ appears at N_{10} of adenine. The water radicals will extract one electron from the N_{10} position of adenine. In the presence of cysteamine, one electron of cysteamine can be supplied to the N_{10} position in which the amino radical ·NH is formed by the extractive reaction of water radical. Table 3.2 lists the reaction indices of the imino form of adenine radical. The adenine radical is more stable than the original adenine.

The chemical repair process of cysteamine for adenine may be represented as follows.

$$\underset{\text{(Extraction)}}{A^{NH_2} + O{\cdot}H \rightarrow A^{{\cdot}NH} + H_2O} \quad \underset{\text{(Repair)}}{+ RSH \rightarrow A^{NH_2} + RS{\cdot}} \tag{3.4}$$

Fig.3.4 shows the radiation protection factor F of cysteamine for adenine. The protection factor for adenine, different from that for cytosine, depends considerably on dose.

As shown in Fig.3.1, the fraction of residual adenine concentration P exceeds unity in a dense cysteamine solution ($x>3\sim6$). As in the case of cytosine, some other compounds which have much higher absorbance than the original π-system of adenine may be formed. Amino group at N_{10} of adenine may be changed to imino group at N_{10}-C_6 bond of adenine.

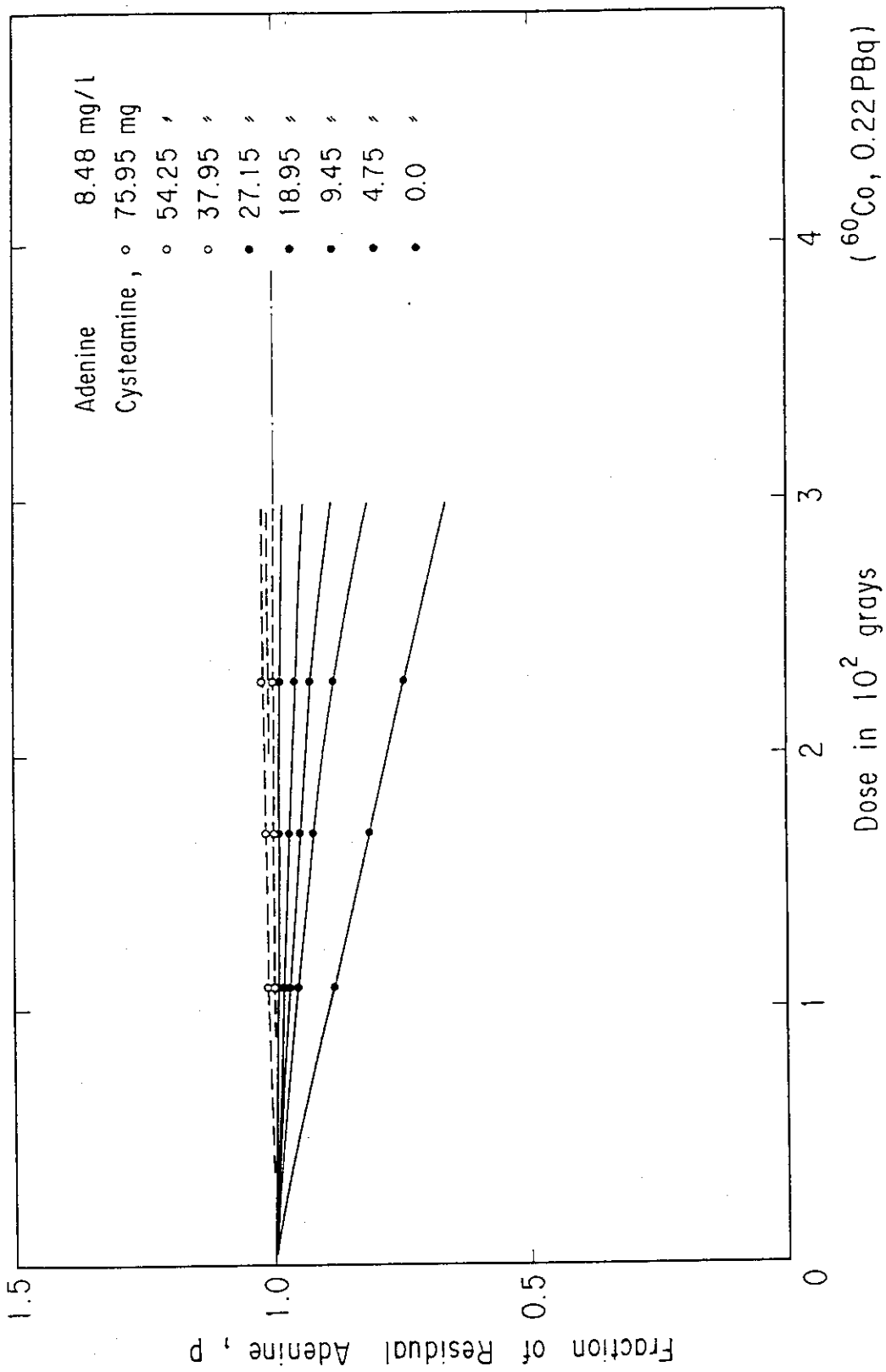


Fig. 3.1 Fraction of residual to initial concentration of adenine in aqueous solutions containing cysteamine, 24hr post irradiation.

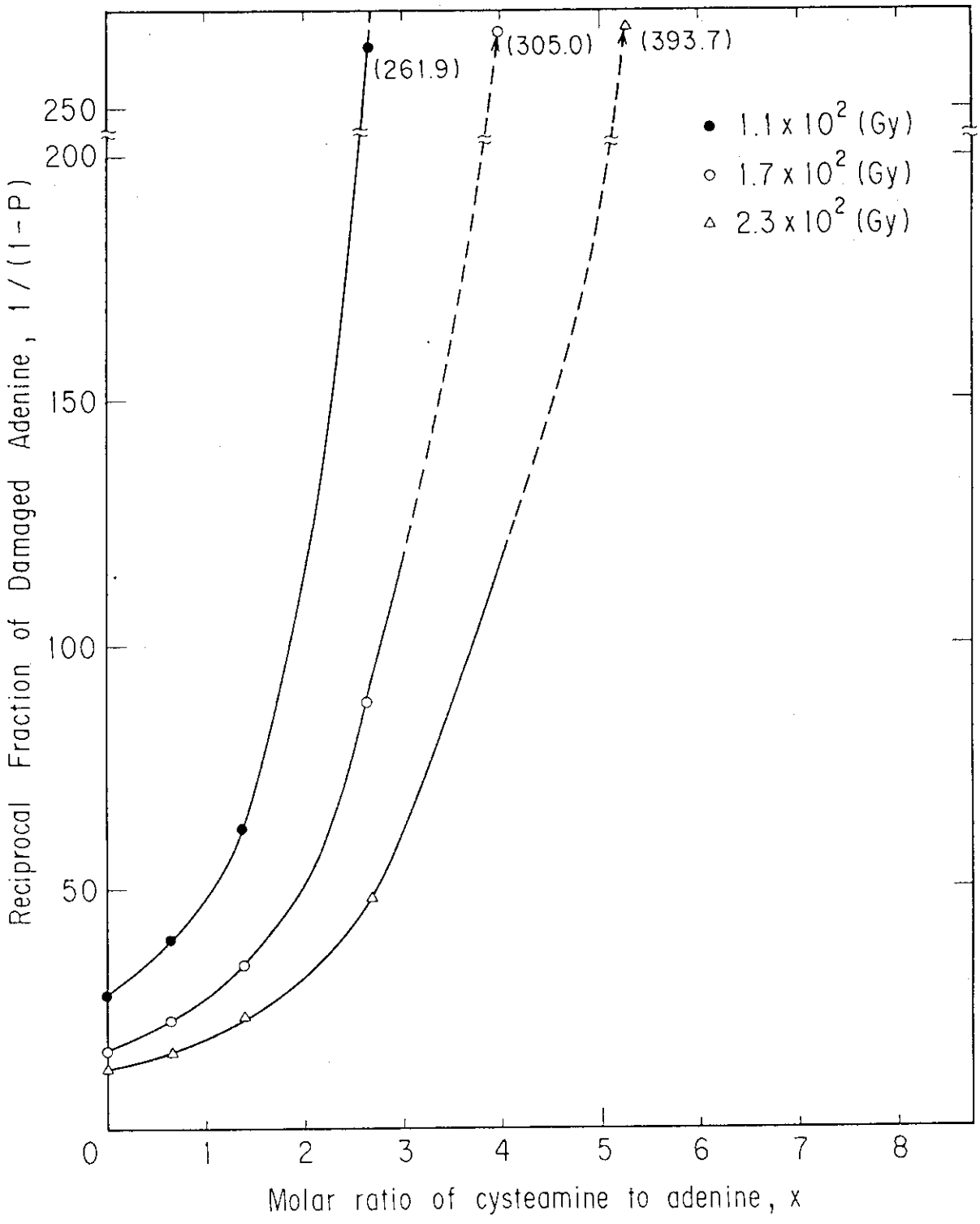


Fig. 3.2 Reciprocal fraction of damaged adenine, as a function of molar ratio of cysteamine to adenine.

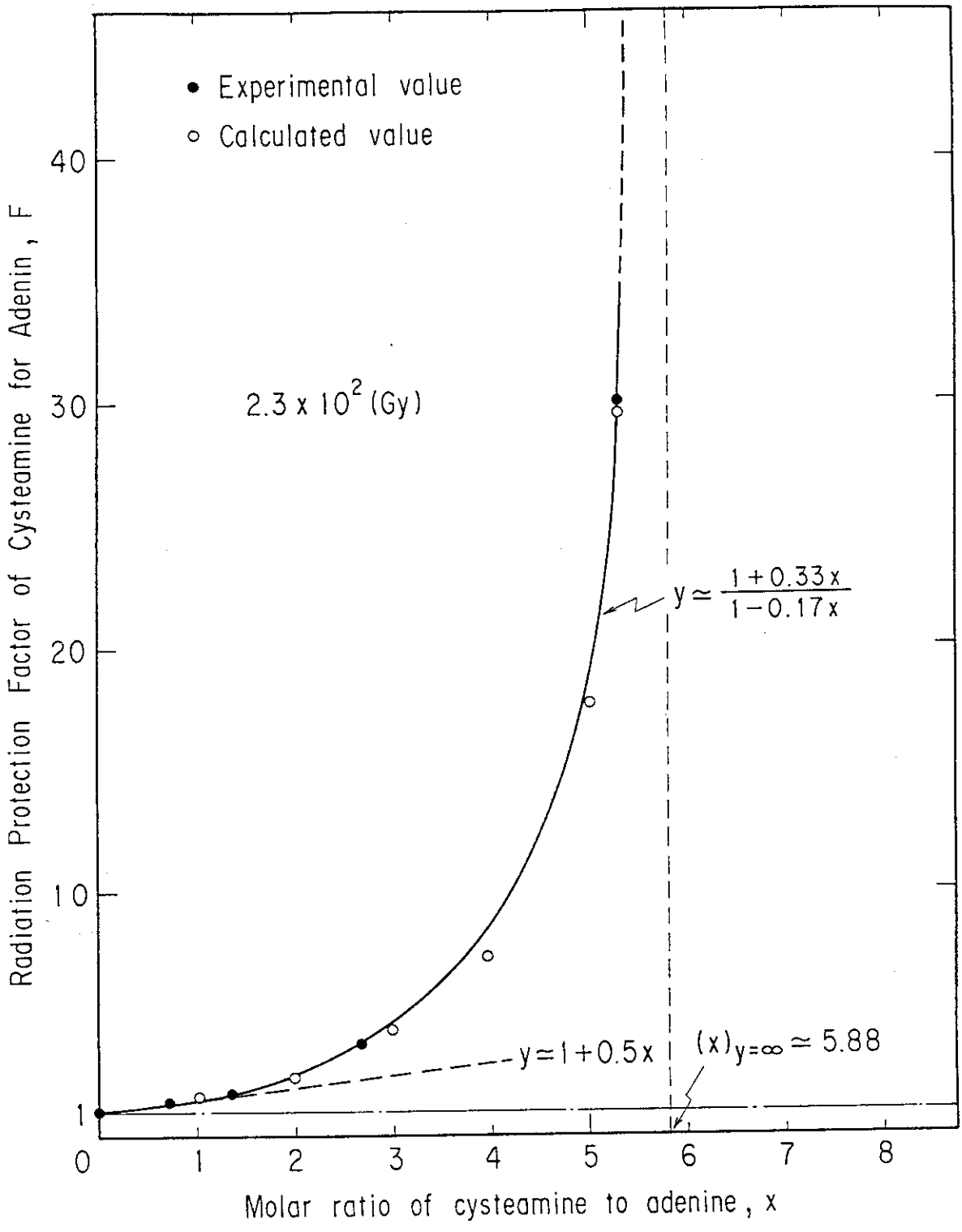
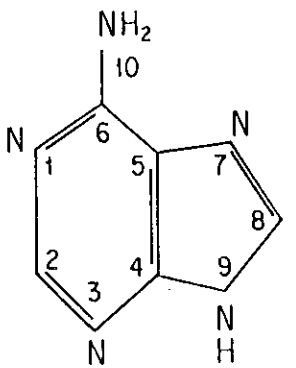


Fig. 3.3 The calculated radiation protection factor of cysteamine for adenine in aqueous solution.

Table 3.1 The reaction indices of adenine, S_r*.

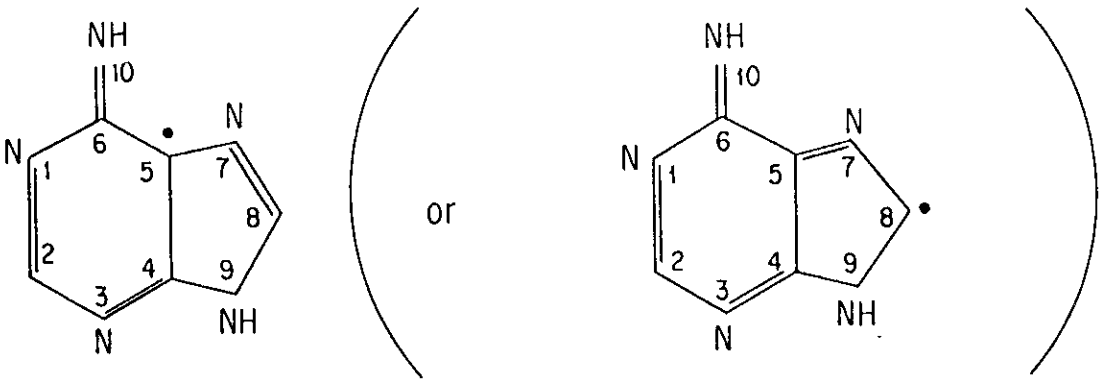


Superdelocalizabilities :

	1	2	3	4	5	6	7	8	9	10
$S_r^{(E)}$	0.7149	0.5938	0.8644	0.9388	1.2714	0.7760	1.5022	0.7510	0.7932	3.9786
$S_r^{(N)}$	0.7211	1.1608	0.5297	0.9757	0.5441	0.9352	0.8088	0.9694	0.9020	0.1578
$S_r^{(R)}$	0.7180	0.8733	0.6970	0.9572	0.9077	0.8566	1.1555	0.8602	0.8476	2.0682

* HMO calculations were done using parameters suggested by T. Yonezawa, et al. (5).

Table 3.2 The reaction indices of adenine radical, S_r*.



Superdelocalizabilities :

	1	2	3	4	5	6	7	8	9	10
$S_r^{(E)}$	0.7188	0.8222	0.9344	0.7122	0.5997	1.0494	1.2333	0.5935	0.6460	1.4205
$S_r^{(N)}$	1.2497	1.1093	0.8049	1.0557	0.9922	0.6372	0.4945	0.9914	0.9232	1.3113
$S_r^{(R)}$	0.9842	0.9657	0.8696	0.8839	0.7960	0.8433	0.8639	0.7925	0.7846	1.3659

* HMO calculations were made on adenine (imino form) π - anion.
One electron was extracted from HOMO of the adenine π - anion.

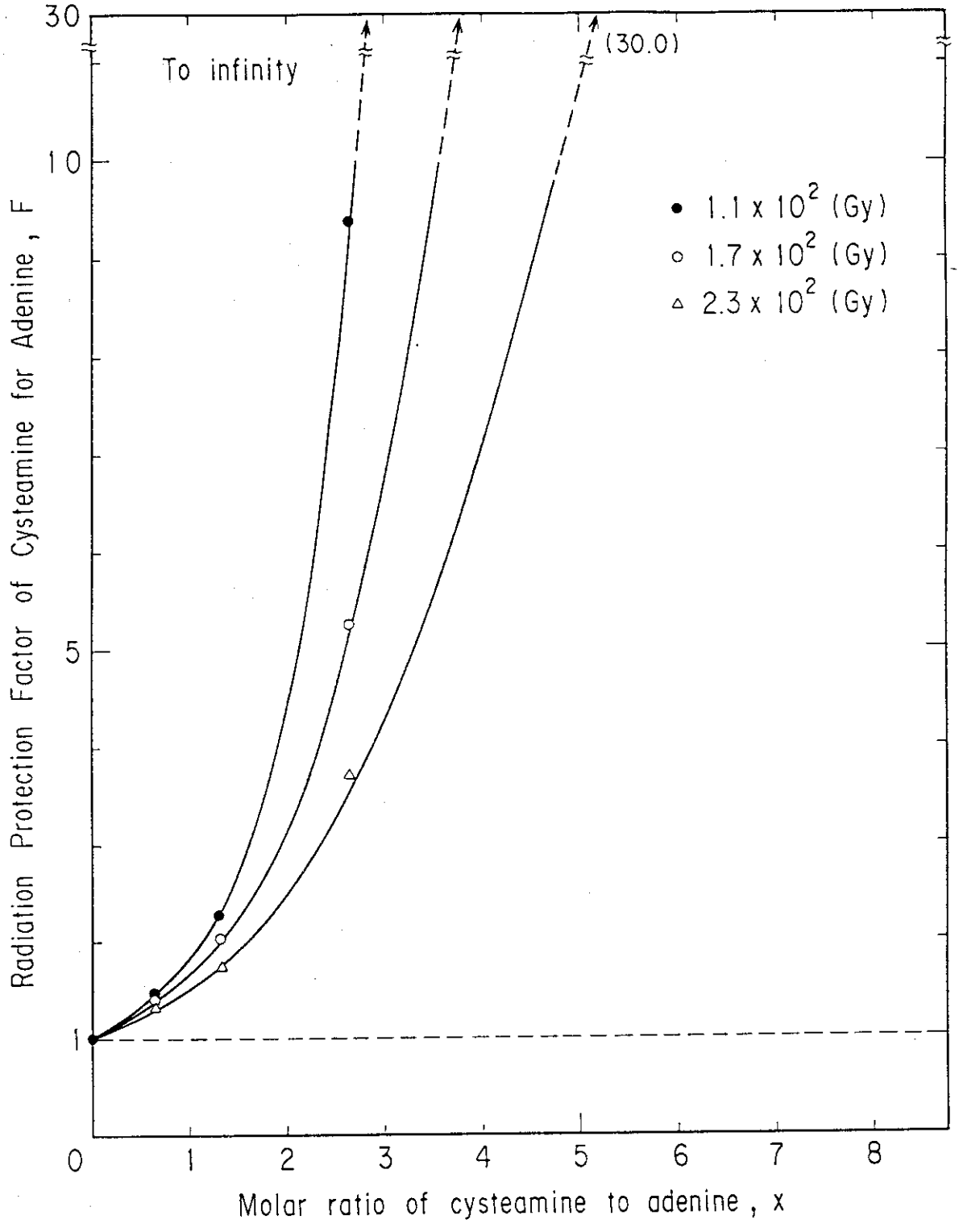


Fig. 3.4 Radiation protection factor of cysteamine for adenine in aqueous solution as a function of molar ratio of cysteamine to adenine.

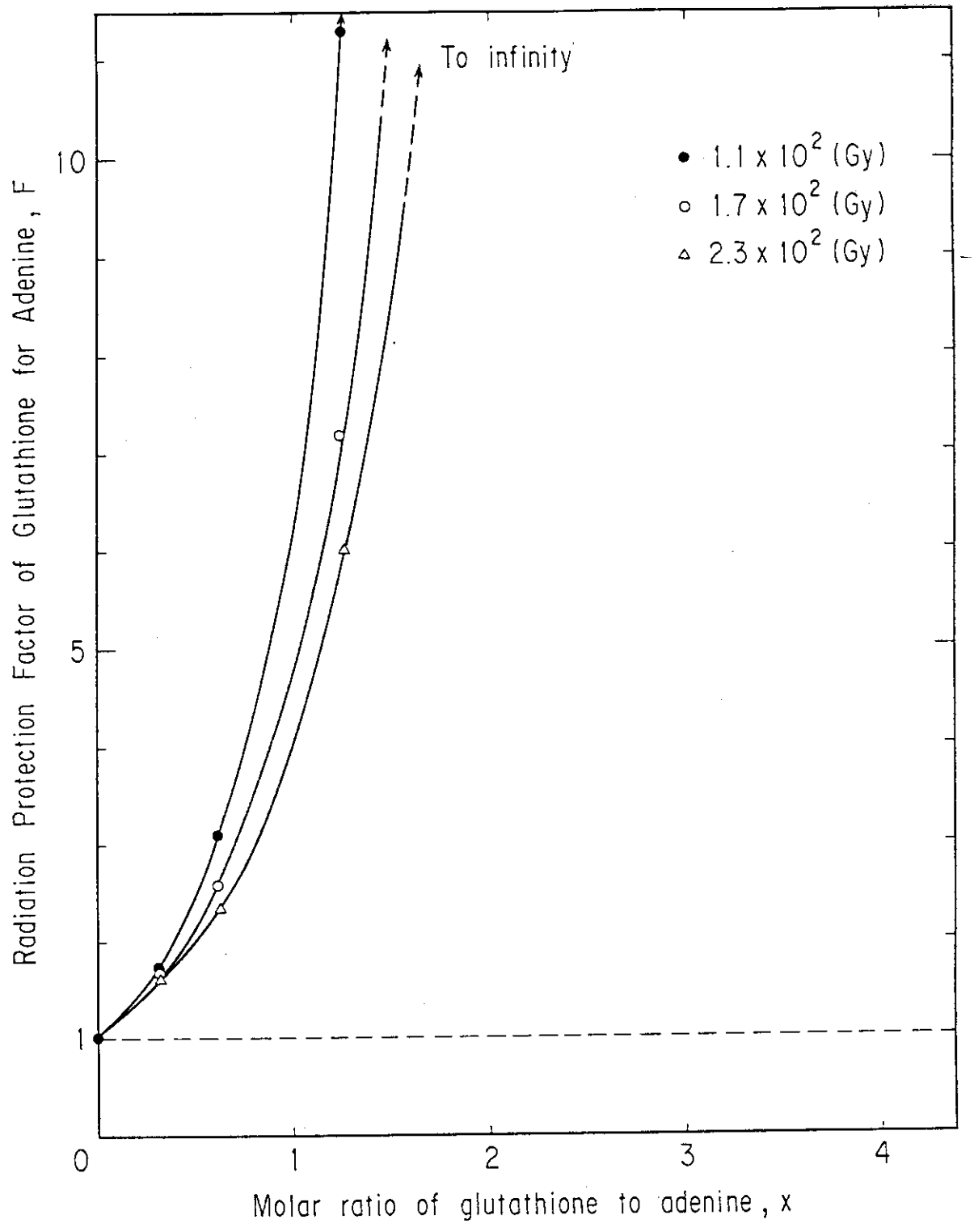


Fig. 3.5 Radiation protection factor of glutathione for adenine in aqueous solution as a function of molar ratio of glutathione to adenine.

$$\mathrm{A}\overset{\mathrm{NH_2}}{} + \mathrm{O{\cdot}H} \xrightarrow[(-H_2O)]{} \mathrm{A}\overset{\mathrm{{\cdot}NH}}{} + \mathrm{RSH} \rightarrow \mathrm{{}^{H}A}\overset{\mathrm{NH}}{} + \mathrm{RS{\cdot}} \qquad (3.5)$$

(Extraction)　(Rearrangement)

However, it is not clear what kinds of compounds are formed by irradiation in dense cysteamine solution.[12][15][16]

Fig.3.5 shows the radiation protection factor F of glutathione for adenine. Glutathione is as good a protective agent for adenine as cysteamine.

3.3 Conclusion.

The above analysis result leads to the following conclusion;

(1)The π-system of one adenine molecule is protected and repaired chemically from the indirect effects of radiation by about three～six cysteamine molecules.

(2)The amino group of adenine releases one electron. The water radicals extract one elecrtron from the amino group in the absence of cysteamine. Intermediate adenine radical which has a amino radical at N_{10} position of adenine is formed.

(3)In the presence of cysteamine, the π-system of the adenine radical is repaired chemically by the supply of one electron from cysteamine to the amino radical, although some kinds of compounds which have much higher absorbance than the original adenine are formed.

The amino group of adenine is electron rich. The amino group located at N_{10} position of adenine protects the π-system of adenine itself from the radiation damage by releasing one electron, as in the case of cytosine.

4. The π-system of guanine.

4.1 Results.

The fraction of residual to initial concentration of guanine, P is shown in Fig.4.1 as a function of total dose. Curves obtained

correspond to cysteamine concentrations added to guanine solution. Residual guanine concentrations were changed gradually post irradiation. In Fig.4.2 curves show the changes in reciprocal fraction of damaged guanine concentration, 1/(1-P) as a function of molar ratio x of cysteamine to guanine. The characteristic curve in the figure means that the secondary reaction of superoxide radical with guanine takes place in dense cysteamine solution, as will be discussed below.

4.2 Analysis and Discussion.

As in the case of thymine, the primary reaction probability of water radicals with guanine, W_G, in a solution with molar ratio x of cysteamine to guanine can be written as follows.

$$(W_G)_x = \frac{n(x)K_G[G]}{n(x)k_G[G] + k_{SH}[SH]}$$

where, k_G is the average rate constant for water radical reaction to break the π-system of guanine. [G] is the initial guanine concentration, k_{SH} is the rate constant of water radical reaction with cysteamine, and [SH] is the initial cysteamine concentration. n(x) is the average number of water radicals to break the π-system of guanine in a solution with molar ratio x of cysteamine to guanine.

To explain the variation of curve in Fig 4.2, it needs to introduce the secondary reaction, in which some fraction of cysteamine radicals formed by the primary reaction react to guanine again. The secondary reaction probability of the cysteamine radical with guanine, W_S, can be written as follows.

$$W_{SH} = \frac{d[SH]}{dt} \Big/ \frac{d[R]}{dt} = \frac{k_{SH}[SH]}{n(x)k_G[G] + k_{SH}[SH]}$$

$$W_S = k_S \cdot W_{SH} = \frac{k_S \cdot k_{SH}[SH]}{n(x)k_G[G] + k_{SH}[SH]} \qquad (4.1)$$

where, W_{SH} is the reaction probability of water radicals with cysteamine per unit time. k_S in Eq(4.1) is defined as the secondary reaction probability of cysteamine radical with guanine. The k_S means that cysteamine radicals accumlated by irradiation react again with the π-system of guanine through the secondary reaction. The k_S may

depend on many parameter, such as the [SH], the [G], the [R], the dissolved oxygen concentration in water and the time post irradiation, also.

Then, the net concentration of damaged guanine by the indirect effects, ΔG_{ID}, can be represented as follows.

$$\Delta G_{ID} = \Delta G_P + \Delta G_S$$

$$= \frac{[R]}{n(x)} \left(\frac{n(x)k_G[G]}{n(x)k_G[G] + k_{SH}[SH]} + \frac{k_S . k_{SH}[SH]}{n(x)k_G[G] + k_{SH}[SH]} \right)$$

where, $x = [SH]/[G]$.

ΔG_P is the damaged guanine concentration by the primary reaction of water radicals, and ΔG_S is the damaged guanine concentration by the secondary reaction of cysteamine radicals. Then,

$$\frac{1}{\Delta G_{ID}} = \frac{n(x)}{[R]} \frac{n(x)k_G[G] + k_{SH}[SH]}{n(x)k_G[G] + k_S \cdot k_{SH}[SH]} . \qquad (4.2)$$

The radiation protection factor of cysteamine for guanine, F can be written as follows,

$$F = \frac{n(x)}{n(0)} \frac{1 + kx}{1 + k_S \cdot kx}$$

$$\sim \frac{1 + k'x}{1 + k_S \cdot kx} , \qquad (4.3)$$

where, $k = k_{SH}/k_G$,

$n(x) = n(0)(1 + k_n x)$ in dilute cysteamine solution, and

$\frac{n(x)}{n(0)}(1 + k_n/n(x)) \sim 1 + k'x$,

$k' = k_n + k/n(0)$,

are assumed, as in the case of adenine.

Fig.4.3 shows the calculated radiation protection factor F, as a function of molar ratio x of cysteamine to guanine. As shown in the figure, the k' and k_S values are obtained in 1.97 and 0.086, respectively.

$$F = y \sim \frac{1 + 1.97x}{1 + 0.17x} ,$$

$k' \sim 1.97$,

$k_S \sim 0.086$.

where, k = k' is assumed in a dense cysteamine solution with x>4 to estimate the k_S value in Eq(4.3).

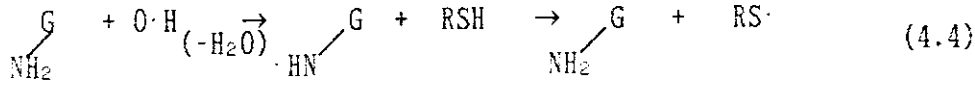
Eq(4.3) means that if the molar ratio x is increased to infinite, the F value is saturated to $1/k_S$.

$$F_{x=\infty} = 1/k_S \sim 11.6$$

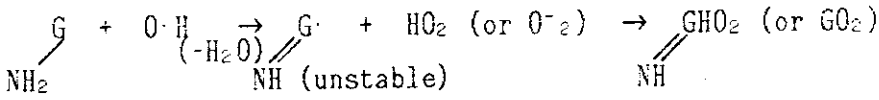
The data at 24 hr post irradiation are used for the calculation of the protection factor F. The secondary reaction of cysteamine radical with guanine takes place slowly in irradiated solution. Guanine radical produced by the primary reaction of water radicals is considerably unstable.[12][16][17][18] Superoxide radicals, which are generated by the reaction of cysteamine radical with hydrogen-peroxide, break slowly the π-system of guanine.

Table.4.1 lists the reaction indices of guanine, the superdelocalizabilities $S_r^{(R)}$. An water radical extracts one electron from N_1ø position of guanine. Table 4.2 lists the reaction indices of imino form of guanine radical, the $S_r^{(R)}$. The guanine radical is very unstable at it's C_8 position for phil-nucleic reaction.[5]

Cysteamine radical formed by irradiation reacts with the dissolved oxygen, and dessociates hydrogenperoxide accumulated in aqueous solution. As the results, the cysteamine radical generates newly superoxide radical. The superoxide radical reacts slowly with the unstable guanine radical. Then, the repair reaction of cysteamine and the secondary reaction of superoxide radical may take place in irradiated solution, as below[12][13].

(1) 

(Extraction) (Repair) (4.4)

(2) 

(Extraction) (Addition:Rupture of the π-system) (4.5)

Fig.4.4 shows the radiation protection factor F of cysteamine for guanine. As shown in the figure, the protection factor is saturated with increasing molar ratio x. Fig.4.5 shows the radiation protection

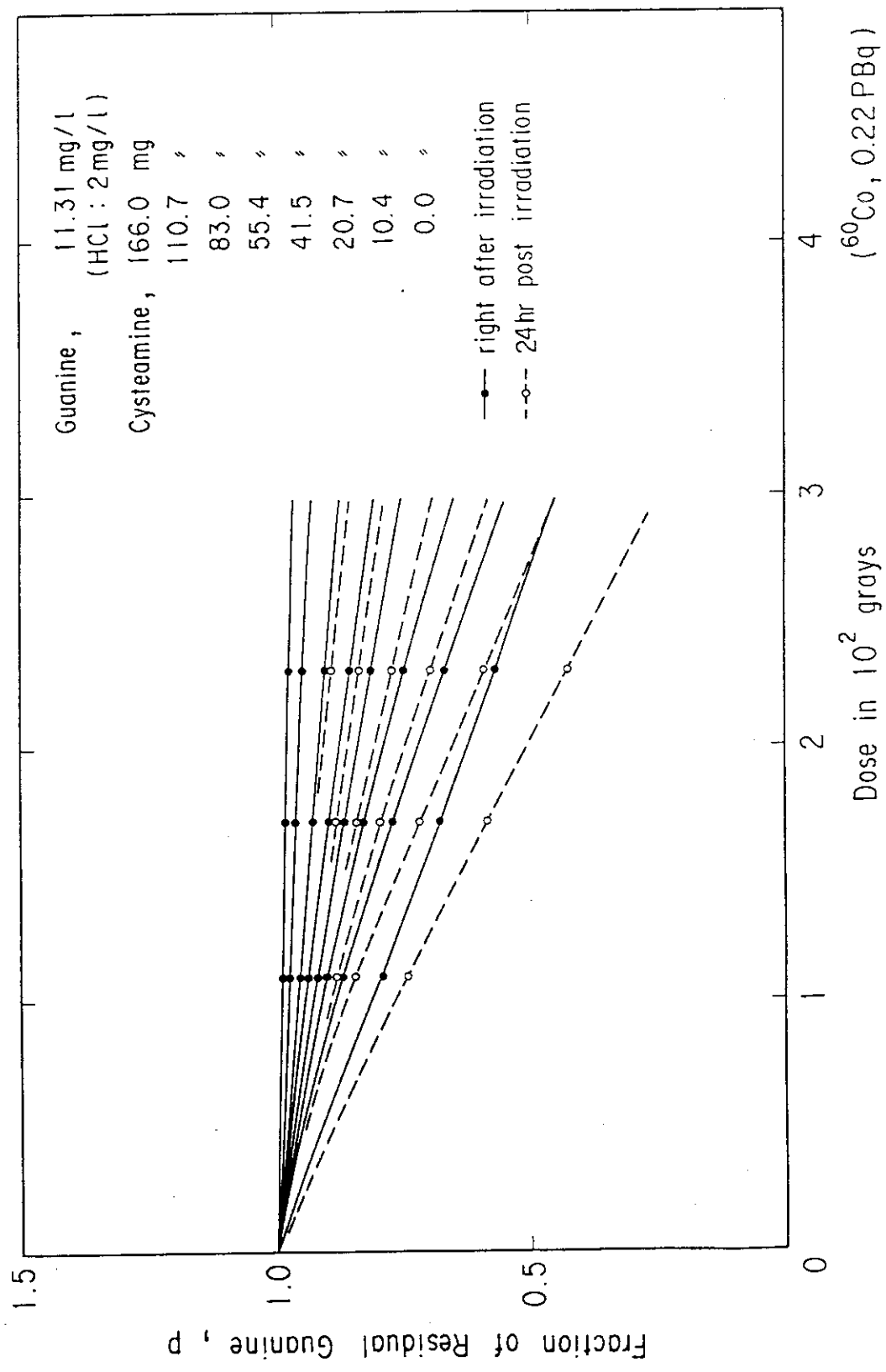


Fig. 4.1 Fraction of residual to initial concentration of guanine in aqueous solutions containing cysteamine.

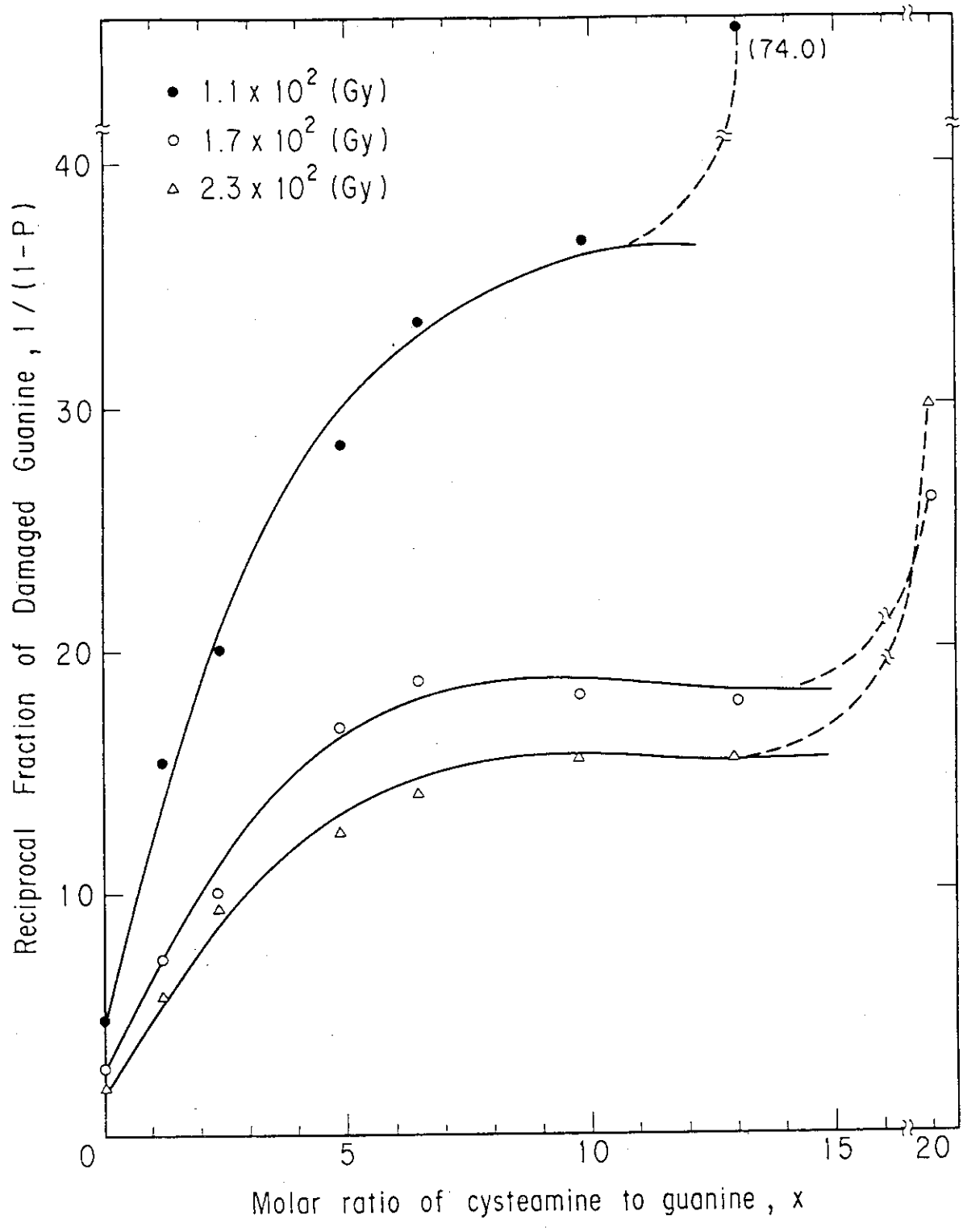


Fig. 4.2 Reciprocal fraction of damaged guanine, as a function of molar ratio of cysteamine to guanine.

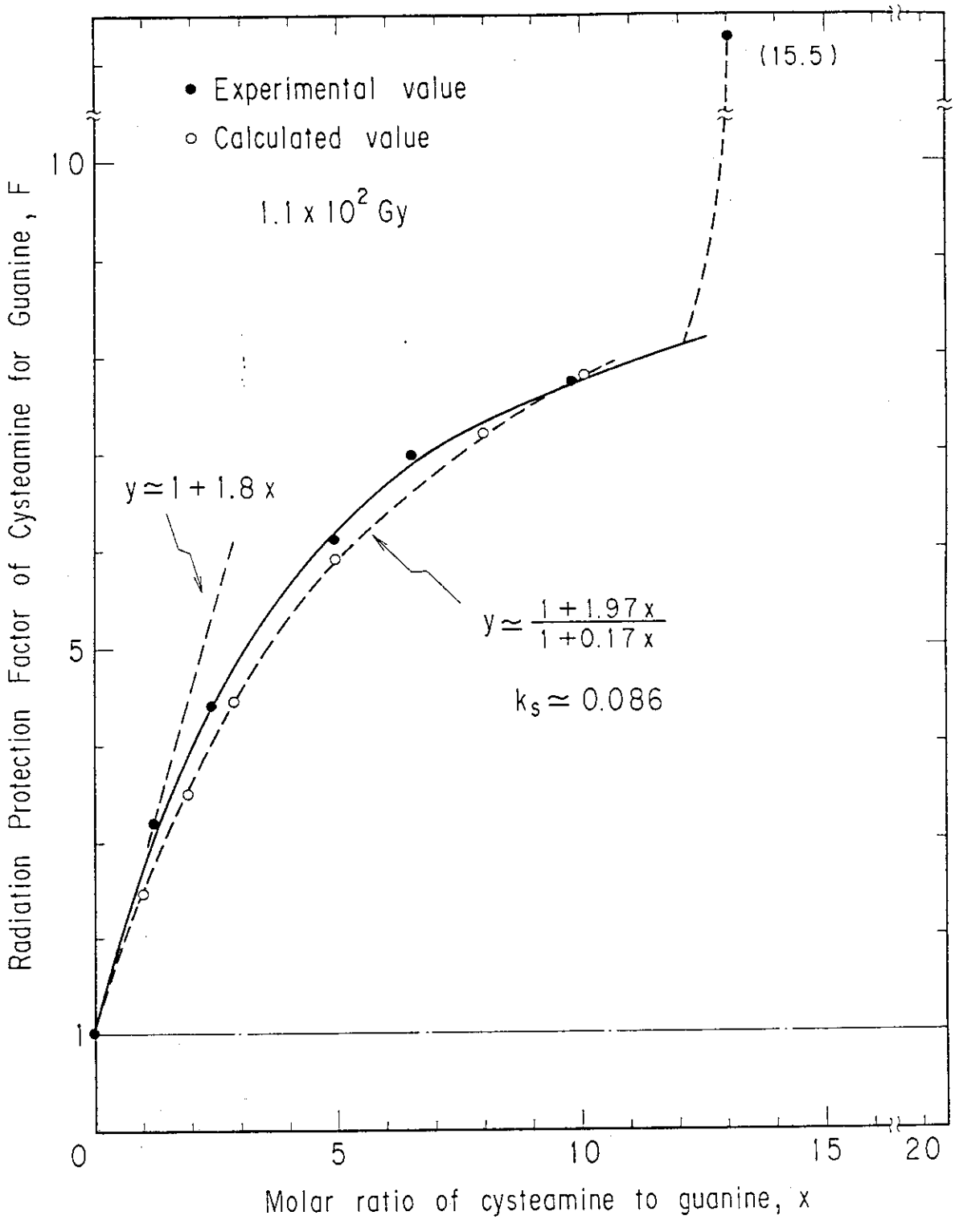
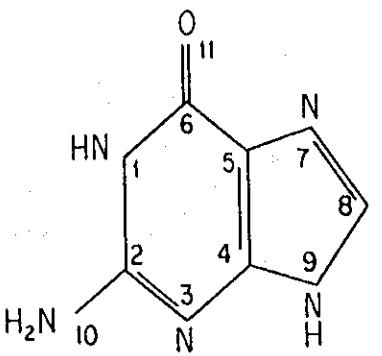


Fig. 4.3 The calculated radiation protection factor of cysteamine for guanine in aqueous solution.

Table 4.1 The reaction indices of guanine (lactum) , S_r*.

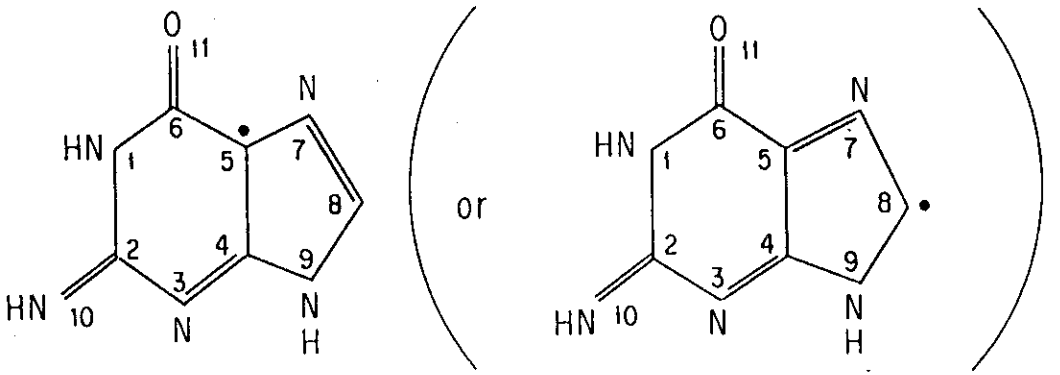


Superdelocalizabilities :

	1	2	3	4	5	6	7	8	9	10	11
$S_r^{(E)}$	0.8984	0.8874	1.1436	0.5115	1.7776	1.0469	1.1338	0.7363	0.7213	3.9280	0.6378
$S_r^{(N)}$	0.7177	1.2292	0.3406	0.9036	0.5975	0.8605	0.4084	0.9269	0.7484	0.1399	0.4407
$S_r^{(R)}$	0.8080	1.0583	0.7421	0.7075	1.1875	0.9537	0.7711	0.8316	0.7348	2.0339	0.5392

* HMO calculations were done using parameters suggested by T. Yonezawa , et al. (5).

Table 4.2 The reaction indices of guanine radical, S_r*.



Superdelocalizabilities :

	1	2	3	4	5	6	7	8	9	10	11
$S_r^{(E)}$	0.8085	0.6015	1.1172	0.4690	1.4691	0.9938	1.0609	0.7782	0.6986	0.2621	0.2670
$S_r^{(N)}$	0.7836	1.3460	0.5135	0.8762	0.5693	1.5338	1.0371	1.0318	0.8432	1.1977	0.9874
$S_r^{(R)}$	0.7961	0.9738	0.8153	0.6726	1.0192	1.2638	1.0490	0.9050	0.7709	0.7299	0.6272

* HMO calculations were made on guanine (imino from) π-anion.
One electron was extracted from HOMO of the guanine π-anion.

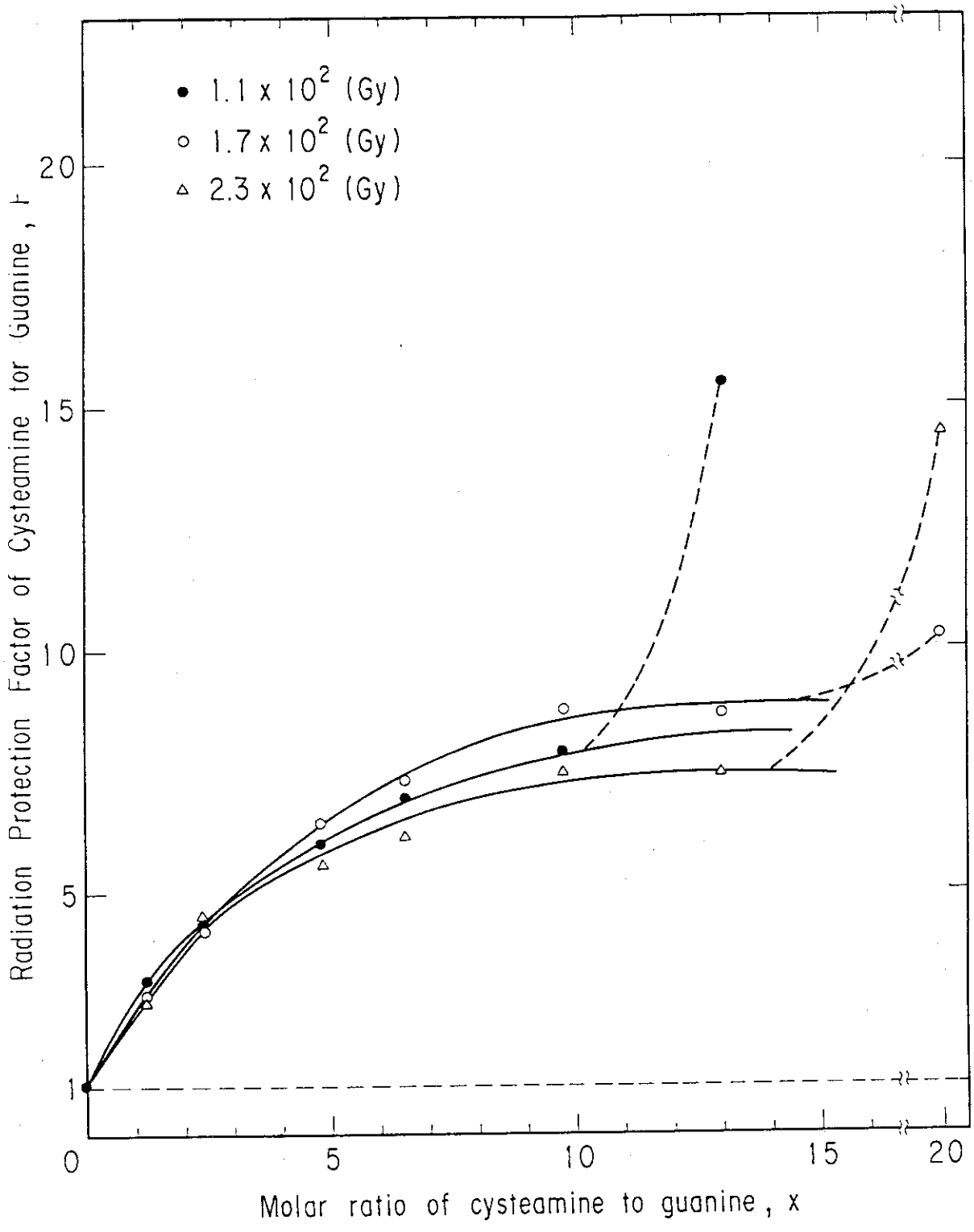


Fig. 4.4 Radiation protection factor of cysteamine for guanine in aqueous solution as a function of molar ratio of cysteamine to guanine.

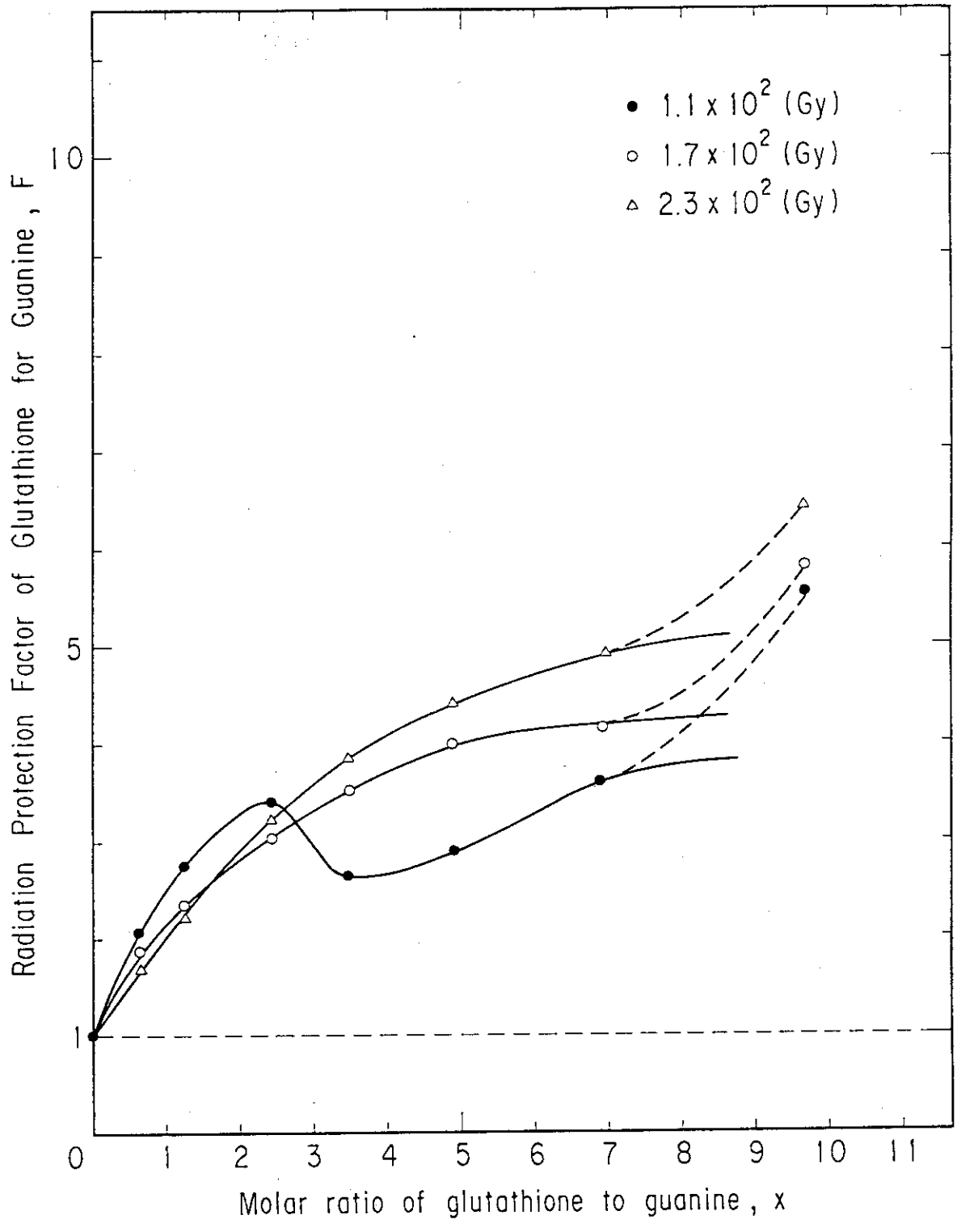


Fig. 4.5 Radiation protection factor of glutathione for guanine in aqueous soluthion as a function of molar ratio of glutathione to guanine.

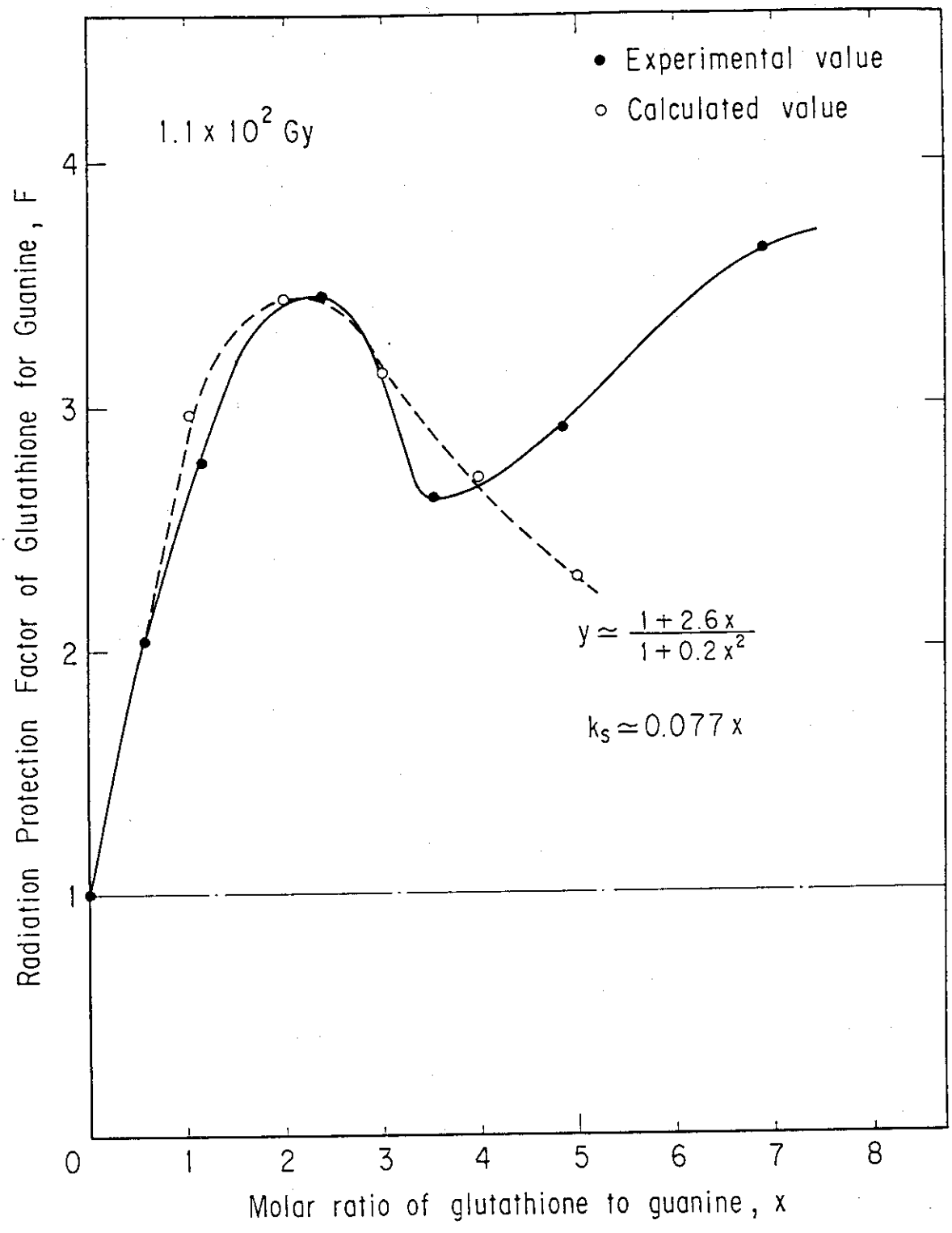


Fig. 4.6 The calculated radiation protection factor of glutathione for guanine, at low dose.

factor F of glutathione for guanine. The protection factor is increased in a dilute glutathione solution, then decreased and saturated with increasing x. Fig.4.6 shows the calculated radiation protection factor at a low dose. The secondary reaction probability, k_s of glutathione radical increases linearly with molar ratio x. Glutathione works as a hydrogenperoxidase in the presence of dissolved oxygen. The secondary reaction of glutathione radical with guanine is higher than that of cysteamine radical.

4.3 Conclusion.

The above analysis result leads to the following conclusion;

(1)The amino group of guanine releases one electron. Amino radical formed at N_{10} of guanine will be repaired by cysteamine. However, guanine radical which has amino radical at the N_{10} position is very unstable and reacts with the dissolved oxygen and/or superoxide radicals.

(2)The π-system of the guanine radical is easily broken by the additive reaction of superoxide radical generated by the reaction of cysteamine radical with hydrogenperoxid.

(3)In a dense cysteamine solution with molar ratio x>4, the protection factor of cysteamine for guanine is saturated with increasing molar ratio x. The protection factor of glutathione for guanine is smaller than that of cysteamine.

The π-systems of cytosine and adenine are protected and repaired by cysteamine from the radiation damage in aqueous solution. The π-systems of thymine and guanine are protected, but not repaired by cysteamine. The experimental results correspond to the "two-component model" proposed by Graslund, et al., which hypothesizes that electron gain (reduction, or anion formation) occurs only at thymine, and that electron loss (oxidation, or cation formation) occurs only at guanine [19][20][21]. Each base in the complementary base-pairs of DNA complements still each other on the radiation protective effects of cysteamine/glutathione in aqueous solution.

Acknowledgement.

I wish to express my appreciation to Dr. Yorio Goto and Dr. Eiko Akastu for their continued interest and encouragement during this experiments. I thank Mr. Hisasi Nagayama and Mr.Takasi Okubo for thier excellent irradiation work in Co-60 facility. I thank Mr. Hiroshi Yokoo and Mr. Masaaki Uchida for helpfull comments and advice. And also, I thank Mr.Shuta Suetake for many stimulating discussion on the present work.

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Ⅱ. Radiation Protective Effects of Cysteamine and Glutathione on Ascorbic Acid in Aqueous Solution.

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Abstract: This paper shows the radiation protective effects of cysteamine and glutathione on ascorbic acid in aqueous solution. Ascorbic acid solutions containing cysteamine (or glutathione) in various concentrations were irradiated with gamma-rays from a 0.22 PBq Co-60 source. The residual concentration of ascorbic acid after irradiation was measured by spectrophotometry. The radiation protective effect of cysteamine (or glutathione) on ascorbic acid was small and saturated with increasing cysteamine concentration. A first approximation analysis was made on the radiation protective effect. The autooxidation of ascorbic acid in aqueous solution containing several kinds of antioxidants was measured and analyzed.

抄録。 この論文では水溶液中のアスコルビン酸に対するシステアミンとグルタチオンの放射線防護特性が示されている。濃度の異なるシステアミンを含む一定濃度のアスコルビン酸を０．２２ＰＢｑの^{60}Ｃｏで照射して、その残留濃度を分光光度計で測定した。アスコルビン酸に対するシステアミンの防護効果はかなり小さく、システアミン濃度をいくら増加してもほぼ一定の効果しか示さない。アスコルビン酸に対するシステアミンの放射線防護効果の特性を一次近次的に解析してある。またスパイス等の抗酸化剤をふくむ水溶液中のアスコルビン酸の自動酸化特性についても近次的な解析を試みている。

Keywords: Radiation Protection, Damage, Repair, Radical Scavenger, Autooxidation, Ascorbic acid, Cysteamine, Glutathione, Spices.

Introduction

Ascorbic acid is well known for it's antiscorbutic properties and to be sensitive for oxidation. Chemists use it widely as a chelating and reducing agent. The radiolysis of ascorbic acid in aqueous solution had been studied by many reseachers. However, the mechanism of how electrons are transferred to oxygen or hydrogen peroxide and the subsequent steps in the oxidation process of ascorbic acid are remained obscure. To study the radiolysis of ascorbic acid, ascorbic acid solutions containing cysteamine or glutathione were irradiated.[1][2][3] Ascorbic acid in aqueous solution is oxidized automatically by the dissolved oxygen in water and/or the oxygen absorbed from the air. The autooxidation rate decreases rapidly in the presence of cysteamine in water. The autooxidation rate of ascorbic acid in aqueous solutions containing cysteamine, glutathione, catalase and several kinds of antioxidants, such as papain, horse-radish, turmeric and so on, were measured and analyzed.

The results are presented in this paper.

1. Radiation protective effects of cysteamine and glutathione on ascorbic acid in aqueous solution.

1.1 Experimental section.

Millimollar solutions (20mg/l) of ascorbic acid containing cysteamine and glutathione in various concentrations were irradiated by γ-rays from a 0.22 PBq Co-60 source. Chemicals used were obtained from Wako Junyaku Co.. Ascorbic acid concentration was determined by spectrophotometry; λ_{max} = 258 nm, ε_{258} = 8630.3 $mol^{-1}cm^{-1}$ for ascorbic acid. The irradiation was made at three different dose levels: $8.9x10^1$, $1.4x10^2$ and $2.6x10^2$ Gy.

1.2 Results.

Fig.1.1 shows the fraction of residual to initial concentration of ascorbic acid, P as a function of irradiation dose. Fig.1.2 shows

the reciprocal fraction of damaged to initial concentration of ascorbic acid, 1/(1-P) as a function of molar ratio x of cysteamine to ascorbic acid. Curves in the figure increase with molar ratio x in x= 0～5. However, the curves are saturated with increasing x in the range of x= 5 ～ 15. Fig.1.3 and 1.4 show the P and the 1/(1-P) in the case of glutathione. The variation of 1/(1-P) in Fig.1.4 increases slightly, then decreases and slowly increases again with increasing molar ratio x of glutathione to ascorbic acid.

1.3 Analysis and Discussion.

The radiolysis of ascorbic acid had been reported by many reseachers, Barr and King, Narasinga Rao and T. Sadat Shafai, etc..[1][2][3] According to these reports, the following reaction mechanisms were suggested in oxygen saturated aqueous solution containing ascorbic acid of $[AH_2] > 0.5 \times 10^{-3}$ mol, where $[AH_2]$ represents a given ascorbic acid concentration.

$$AH_2 + OH \rightarrow A\cdot H + H_2O \quad (1.1)$$

$$AH_2 + HO_2 \rightarrow A\cdot H + H_2O_2 \quad (1.2)$$

$$A\cdot H + O_2 \rightarrow AHO_2 \quad (1.3)$$

$$A\cdot H + AHO_2 \rightarrow 2A + H_2O_2 \quad (1.4)$$

These reactions mean that one ascorbic acid molecule forms one dehydroascorbic acid molecule by one water radical species, OH or HO_2 in the presence of oxygen in water.

As shown in Fig.1.2, the reciprocal fraction of damaged ascorbic acid concentration increases very rapidly with molar ratio x in a small x. Then curves are saturated with increasing x. The characteristic variation can be analyzed as below.

The primary reaction probability of water radicals with ascorbic acid, $(W_{AH_2})x$ can be written as follows.

$$(W_{AH_2})x = \frac{d[AH_2]}{dt} \Big/ \frac{d[R]}{dt} = \frac{n(x)k_{AH_2}[AH_2]}{n(x)k_{AH_2}[AH_2] + k_{SH}[SH]}$$

where, k_{AH_2} is the average rate constant of water radical reaction to break the π-system of ascorbic acid, $[AH_2]$ is the initial ascorbic

acid concentration, k_{SH} is the average rate constant of water radical reaction with cysteamine, [SH] is the initial cysteamine concentration, and n(x) is the average number of water radicals to break the π-system of ascorbic acid in a solution with molar ratio x.

Cysteamine radicals are formed by the following reactions.

$$RSH + OH \rightarrow RS\cdot + H_2O \tag{1.6}$$

$$RSH + HO_2 \rightarrow RS\cdot + H_2O_2 \tag{1.7}$$

$$W_{SH} = \frac{d[SH]}{dt} \Big/ \frac{d[R]}{dt} = \frac{k_{SH}[SH]}{n(x)k_{AH2}[AH_2] + k_{SH}[SH]} ,$$

where, W_{SH} is the reaction probability of water radicals with cysteamine per unit time. To explain the characteristic variation of Fig 1.2 and 1.4, it needs to introduce the secondary reaction in which some fraction of cysteamine radicals, RS· react again with ascorbic acid. If the secondary reaction of the cysteamine radical with ascorbic acid takes place during irradiation, the secondary reaction probability, W_S can be written as follows, as in the case of guanine.

$$W_S = k_S \cdot W_{SH} = \frac{k_S \cdot k_{SH}[SH]}{n(x)k_{AH2}[AH_2] + k_{SH}[SH]} .$$

where, k_S is the secondary reaction probability of cysteamine radical with ascorbic acid. The k_S depends on many parameter, such as the [SH], the $[AH_2]$, the [R] and time post irradiation.

Then, the net concentration of damaged ascorbic acid, ΔAH_{2D} can be written as the sum of damaged concentrations, ΔAH_{2P} by the primary reaction and ΔAH_{2S} by the secondary reaction.

$$\Delta AH_{2D} = \Delta AH_{2P} + \Delta AH_{2S}$$

$$= \frac{[R]}{n(x)} \left(\frac{n(x)k_{AH2}[AH_2] + k_S \cdot k_{SH}[SH]}{n(x)k_{AH2}[AH_2] + k_{SH}[SH]} \right)$$

or,

$$\frac{1}{\Delta AH_{2D}} = \frac{n(x)}{[R]} \left(\frac{1 + k\,x}{1 + k_S \cdot k\,x} \right) . \tag{1.8}$$

where, $k = k_{SH}/k_{AH2}$, and $x = [SH]/[AH_2]$.

As in the case of guanine, the protection factor F of cysteamine for ascorbic acid can be written by the following relations.

$$F = \frac{1/(1-P)_0}{1/(1-P)_x} \sim \frac{n(x)}{n(0)} \quad \frac{1 + k\,x}{1 + k_S \cdot k\,x} \sim \frac{1 + k'x}{1 + k_S \cdot kx} , \tag{1.9}$$

where,

$n(x) \sim n(0)(1 + k_n x)$, in a dilute cysteamine solution, and

$$\frac{n(x)}{n(0)}(1 + kx) \sim 1 + k'x ,$$

$$k' \sim k_n + k/n(0),$$

are assumed.

Fig.1.5 and 1.6 show the calculated protection factor F of cysteamine and glutathione at low dose, respectively. The protection factor F of cysteamine for ascorbic acid can be approximated as follows.

If k_s is a constant with x,

$$k_s \sim A ,$$

then,

$$F \sim \frac{1 + k'x}{1 + A \cdot k\, x} \sim \frac{1 + 1.96x}{1 + 1.29x} \quad \text{at } 0<x<5 ,$$

$$\sim \frac{1 + 1.96x}{1 + 1.38x} \quad \text{at } 5<x<15 .$$

As shown in Fig.1.5,

$k' \sim 1.96$, and $k_s \sim 0.661$, at $x < 5$,

~ 0.705. at $5 < x < 15$.

The k_s value means that cysteamine radicals accumlated in water react with ascorbic acid again, by a fraction of 0.661 in a dilute cysteamine solution of $x < 5$, and by a fraction of 0.705 in a dense cysteamine solution of x in $5< x <15$. The difference ΔQ, shown in the figure, seems to correspond to the dissolved oxygen concentration in water. Dense cysteamine solution absorbs much more oxygen from the air than dilute cysteamine solution, through the oxidation of cysteamine itself'. As shown in Fig.1.2, the effects of the dissolved oxygen in water didn't appear at a high dose (1.4×10^2 and 2.6×10^2 Gy).

In the case of glutathione, if k_s increases lineally with x,

or $k_s \sim Ax$, at $x < 1$

then,

$$F \sim \frac{1 + k'x}{1 + A \cdot kx^2} \sim \frac{1 + x}{1 + 1.499x^2} .$$

The k' and the A values can be estimated, as shown in Fig.1.6.

$$k' \sim 1.0, \quad \text{and} \quad A \sim 1.499 ,$$

$$k_S \sim 1.499\, x, \qquad \text{at} \quad x < 1.$$

In the region of $5 > x > 1$, if the k_S is a constant with x,

$$k_S \sim A,$$

$$F \sim \frac{1 + k'x}{1 + A \cdot kx} \sim \frac{1 + x}{1 + 0.883x} .$$

or,

$$k' \sim 1.0 \quad \text{and} \quad k_S \sim 0.883, \quad \text{at} \quad 5 > x > 1.$$

The k' value in the case of cysteamine is about two times higher than that in the case of glutathione. This means that the secondary reaction takes place easier in glutathione solution than in cysteamine solution. Eq(1.9) means that the protection factor F for ascorbic acid is limitted to $1/k_S$, even though cysteamine or glutathione concentration increases in ascorbic acid solution.

$$\lim_{x \to \infty,} F = \frac{1}{k_S} . \tag{1.10}$$

OH radical extracts one electron from ascorbic acid or cysteamine, and forms one water molecule. H and e^-_{aq} radical combine with the dissolved oyxgen in water, and form HO_2 radical. The HO_2 radical extracts one electron from ascorbic acid or cysteamine, and forms one hydrogenperoxide. So a plenty of hydrogenperoxides are accumlated in ascorbic acid solution containing cysteamine or glutathione by irradiation. If one hydrogenperoxide extracts one electron from ascorbic acid, the secondary reaction probability k_S can be estimated as follows,

$$k_S \sim \frac{G(e_{aq}) + G(H) + G(H_2O_2)}{G(\text{radicals})} = \frac{4.0}{6.05} = 0.661.$$

This value is very close to the k_S value obtained in the case of cysteamine, 0.661~0.705. The k_S value, 0.883 in the case of glutathione, however, is very different from the 0.661. The secondary reaction with ascorbic acid takes place easier in glutathione solution than in cysteamine solution.[4]

Table 1.1 lists the reaction indices of ascorbic acid. The largest

value of the superdelocalizabilities, $S_r^{(R)}$ appears at $(OH)_6$ position. The second large value of the $S_r^{(R)}$ appears at $(OH)_5$ position. The water radical, OH or HO_2 extracts one electron from the $(OH)_6$ or the $(OH)_5$ position.[5]

Table 1.2 lists an ascorbate radical, which is extracted one electron from the $(OH)_6$ position of ascorbic acid and is changed to the keto form at C_3-O_6 bond of ascorbic acid. The large values of the $S_r^{(R)}$ appear at C_4 and $(OH)_5$ position of the ascorbate radical.

The water radical reaction is for phil-nucleic at the C_4 position. The dissolved oxygen in water or HO_2 radical adds to the C_4 position. This may correspond to reaction (1.3).

As listed in the table 1.3, dehydroascorbic acid is not a stable molecule. The $S_r^{(R)}$ values are considerably large at position C_2, C_3, and C_4. The water radicals may react with these positions, and break the π-system of dehydroascorbic acid.

The following chain reaction mechanism can be considered as a secondary reaction of cysteamine radical.

$$RS\cdot + HO_2 \rightarrow RSOOH \qquad (1.11)$$

$$RSOOH + H_2O_2 \rightarrow RSO\cdot + H_2O + HO_2 \qquad (1.12)$$

As a result, one hydrogenperoxid H_2O_2 is changed to a water molecule, and another HO_2 and an oxidant radical $RSO\cdot$ are produced newly in water. The chain reaction holds untill the hydrogenperoxide is consumed.

$$RS\cdot + H_2O_2 \rightarrow RSO\cdot + H_2O \qquad (1.13)$$

The cysteamine radical and the hydrogenperoxide accumlated by irradiation generate another oxidant radical $RSO\cdot$, which extracts one electron from ascorbic acid or cysteamine, again.[6][7][8]

$$AH_2 + RSO\cdot \xrightarrow{(k_5)} A\cdot H + RSOH\ ,$$
$$AH_2 + HO_2 \rightarrow A\cdot H + H_2O_2\ . \qquad (1.14)$$

It can be considered that the chain reaction of $RS\cdot$ and H_2O_2 is a fundamental reaction mechanism of SH compound in the presence of oxygen in water. The SH compound works as a peroxidase.

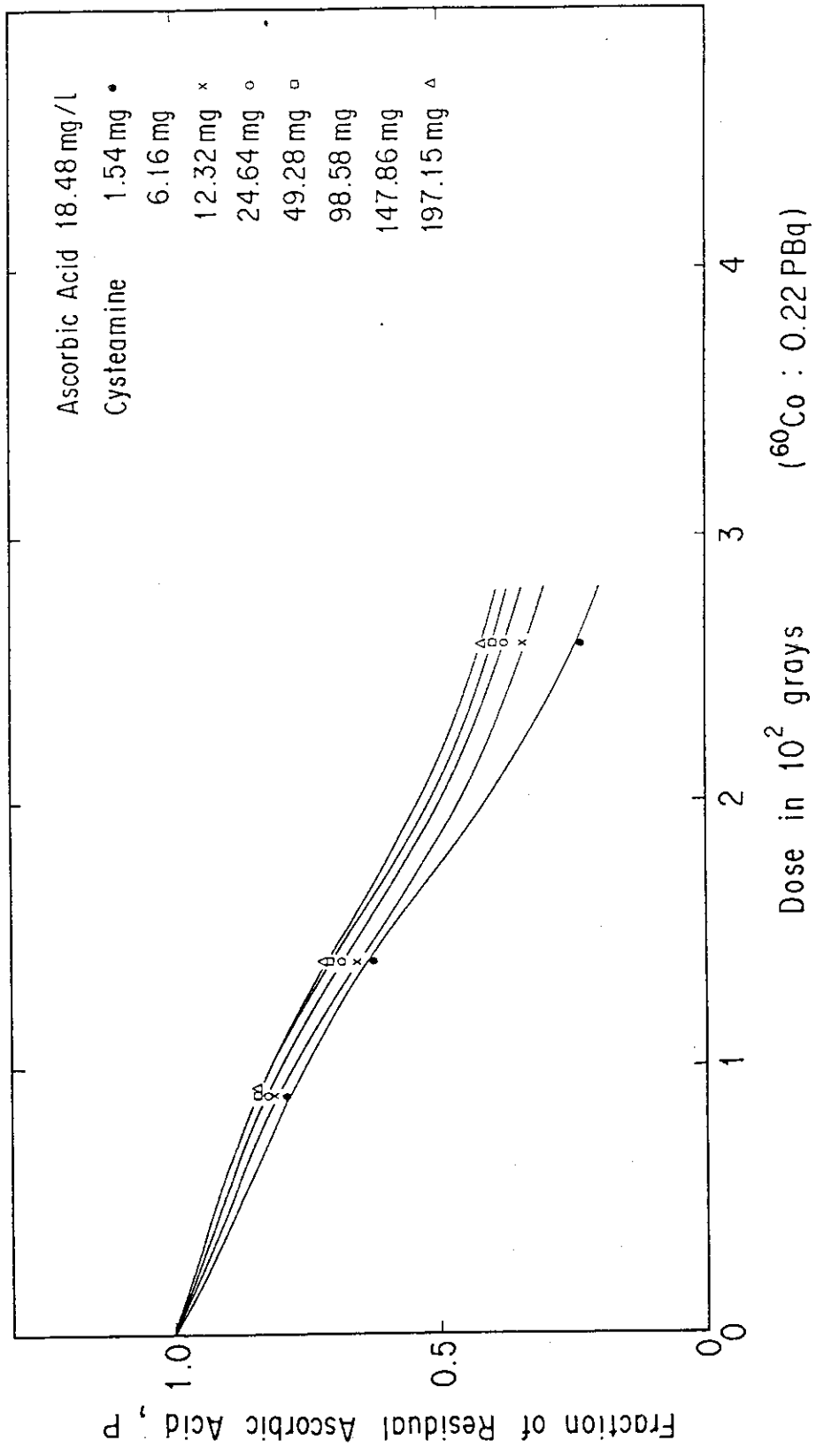


Fig. 1.1 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing cysteamine, right after irradiation.

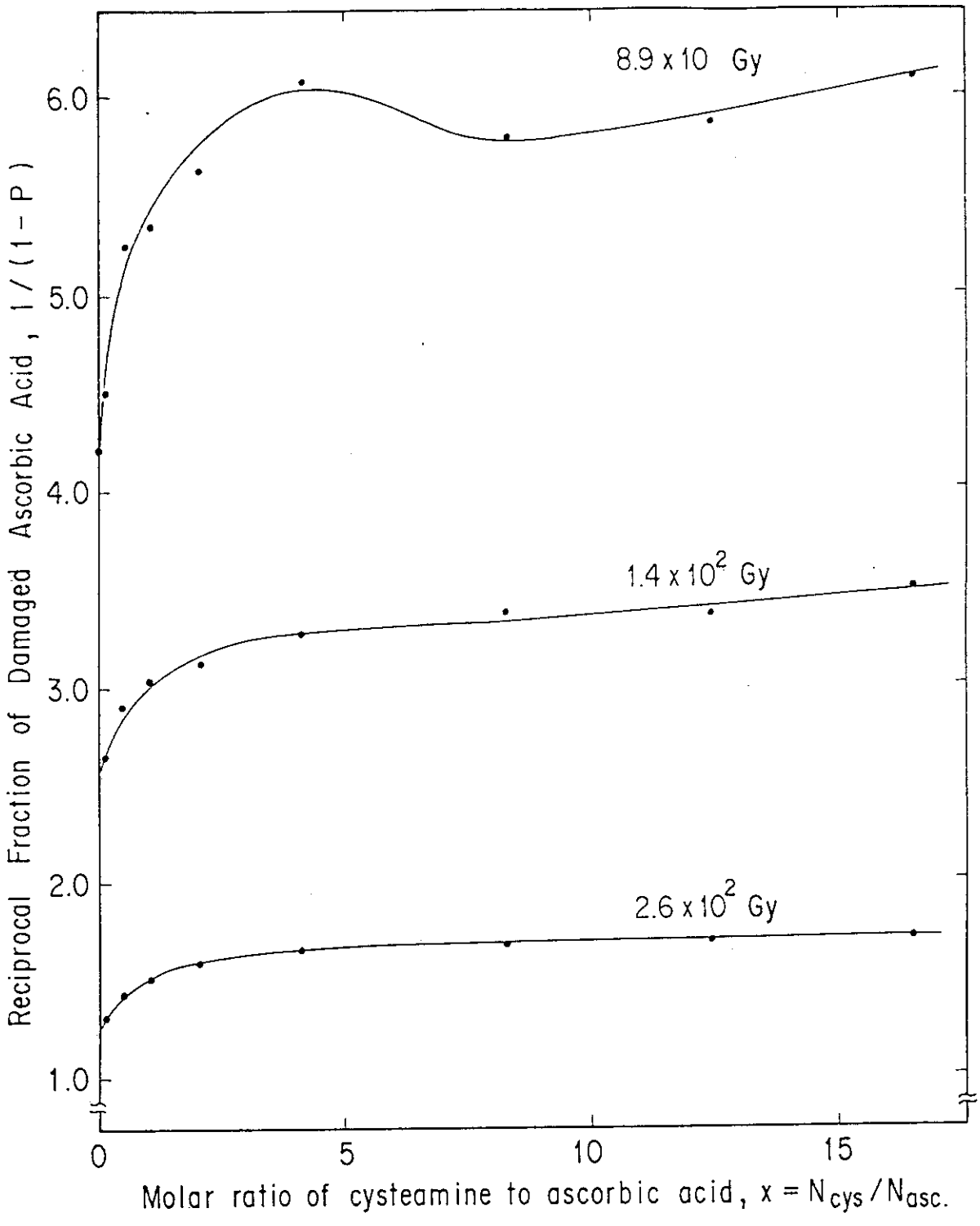


Fig. 1.2 Reciprocal fraction of damaged ascorbic acid, as a function of molar ratio of cysteamine to ascorbic acid.

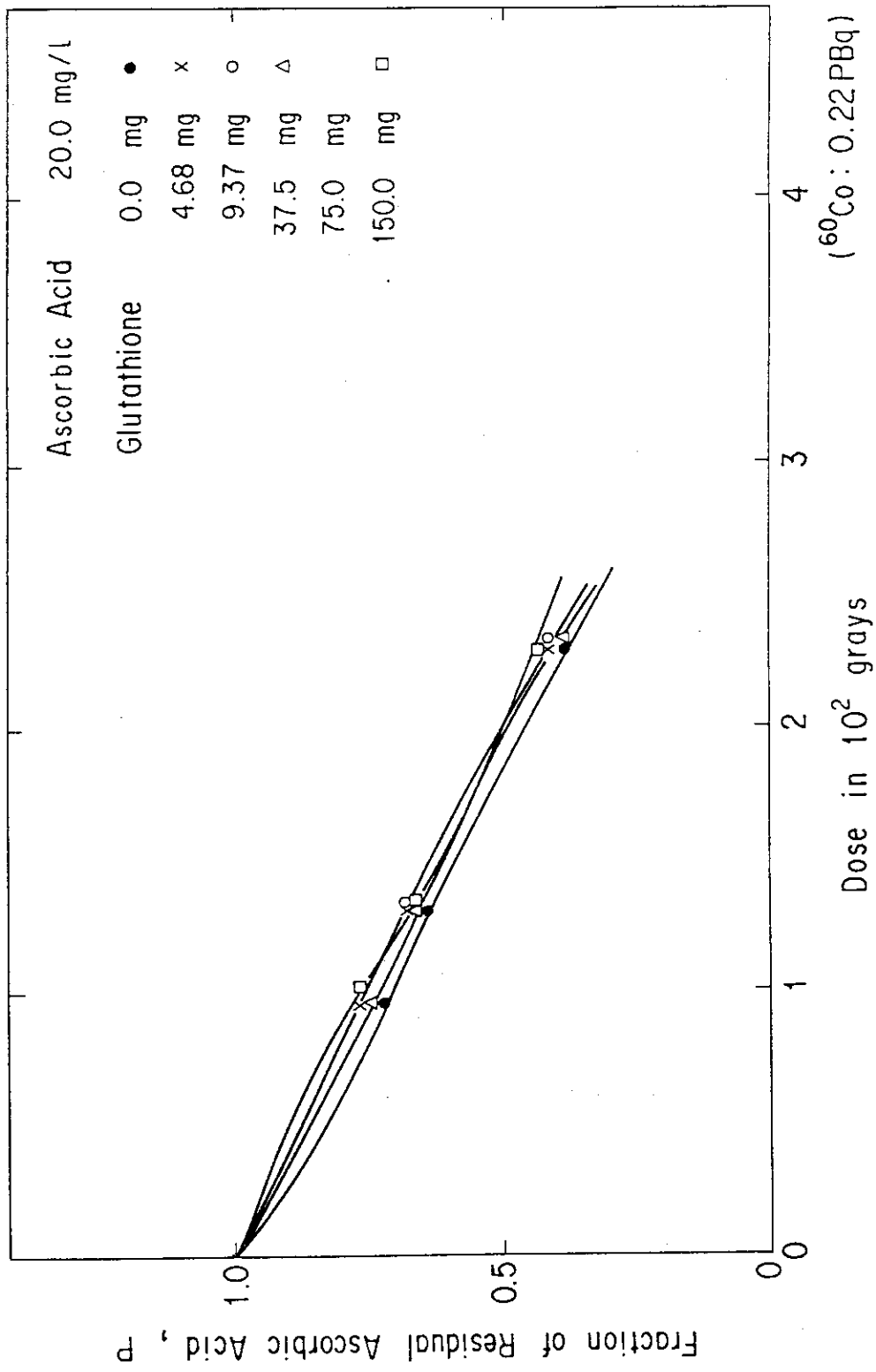


Fig. 1.3 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing glutathione (reduced form) , right after irradiation.

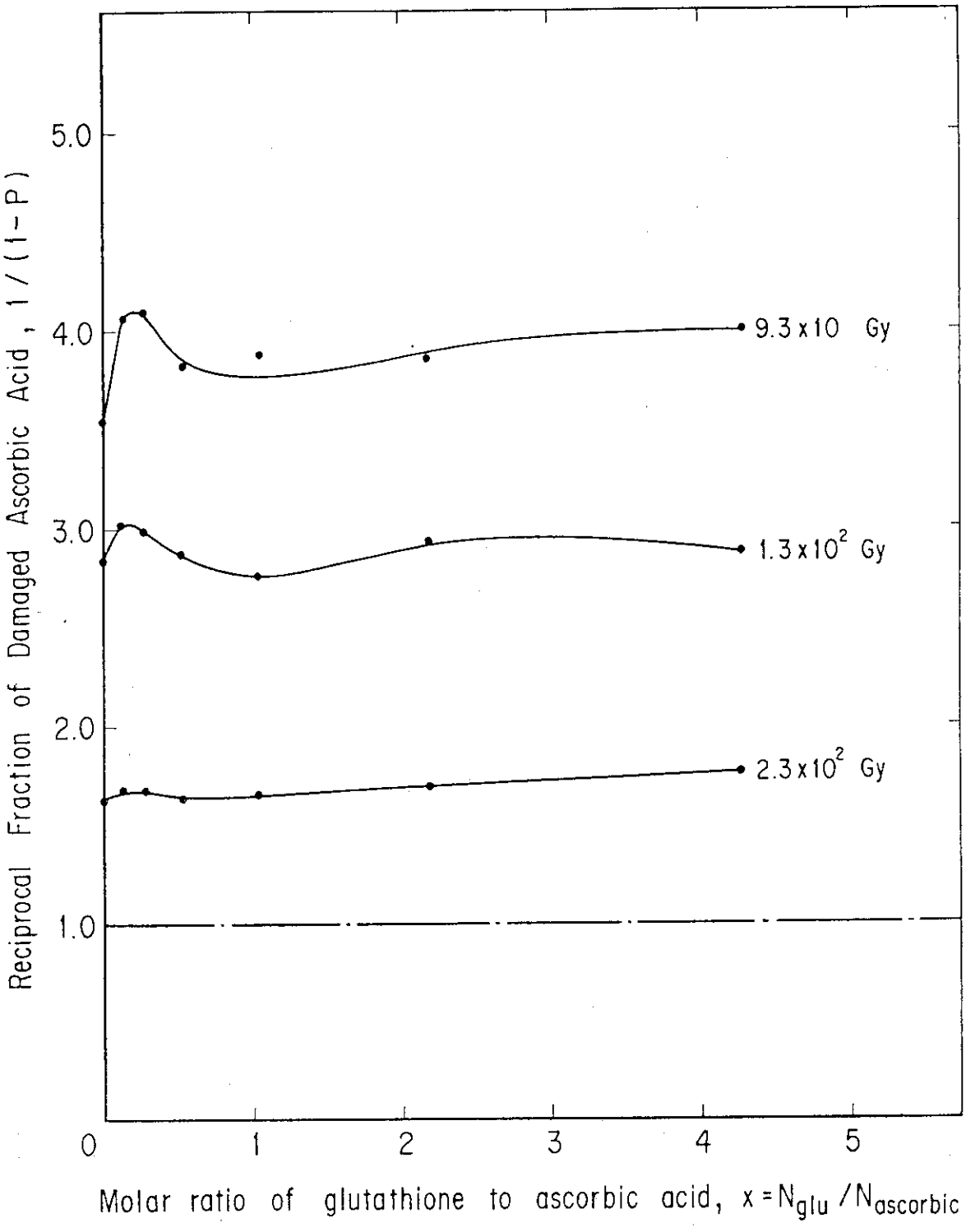


Fig. 1.4 Reciprocal fraction of damaged ascorbic acid, as a function of molar ratio of glutathione to ascorbic acid.

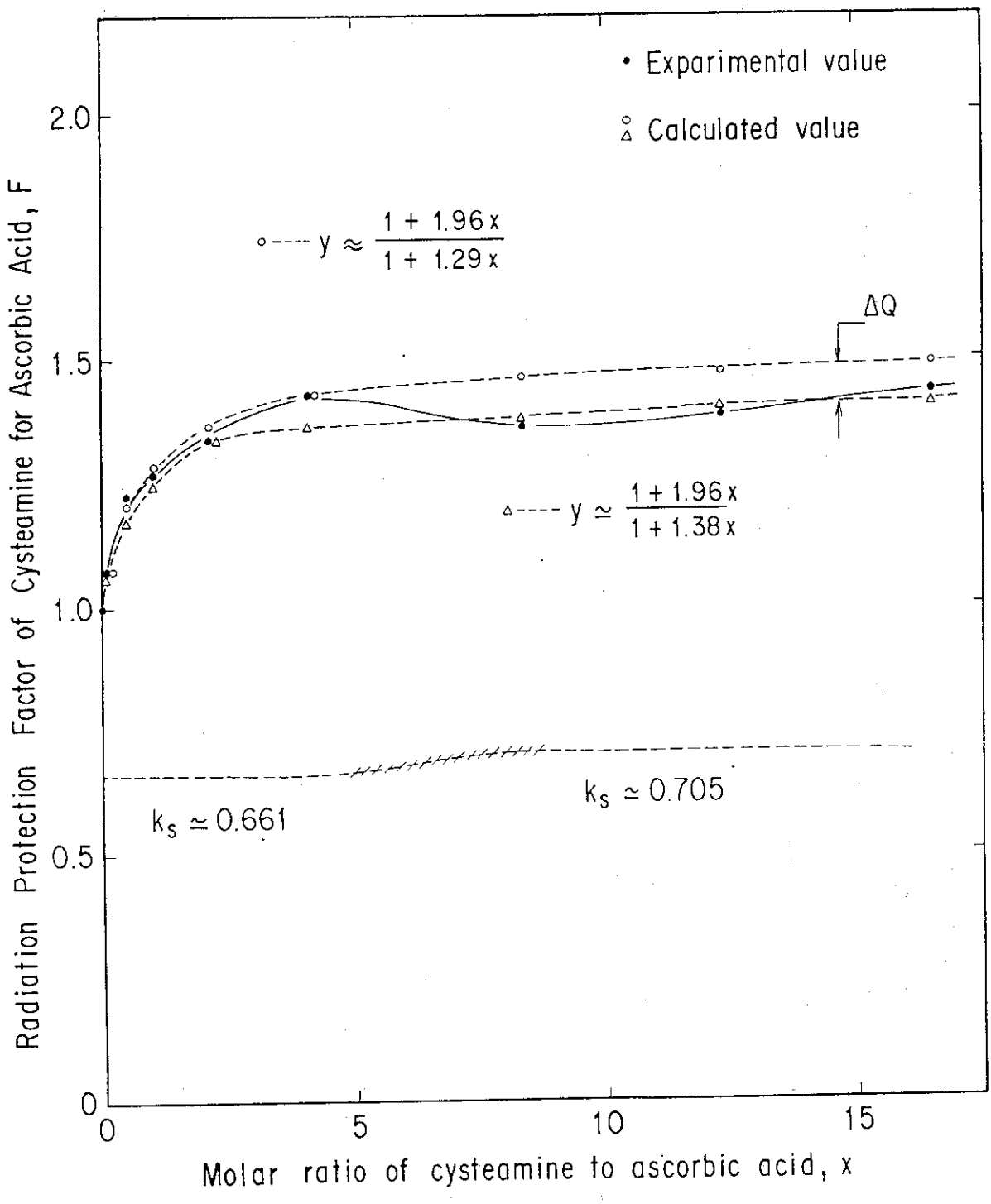


Fig. 1.5 The calculated radiation protection factor of cysteamine for ascorbic acid, at low dose.

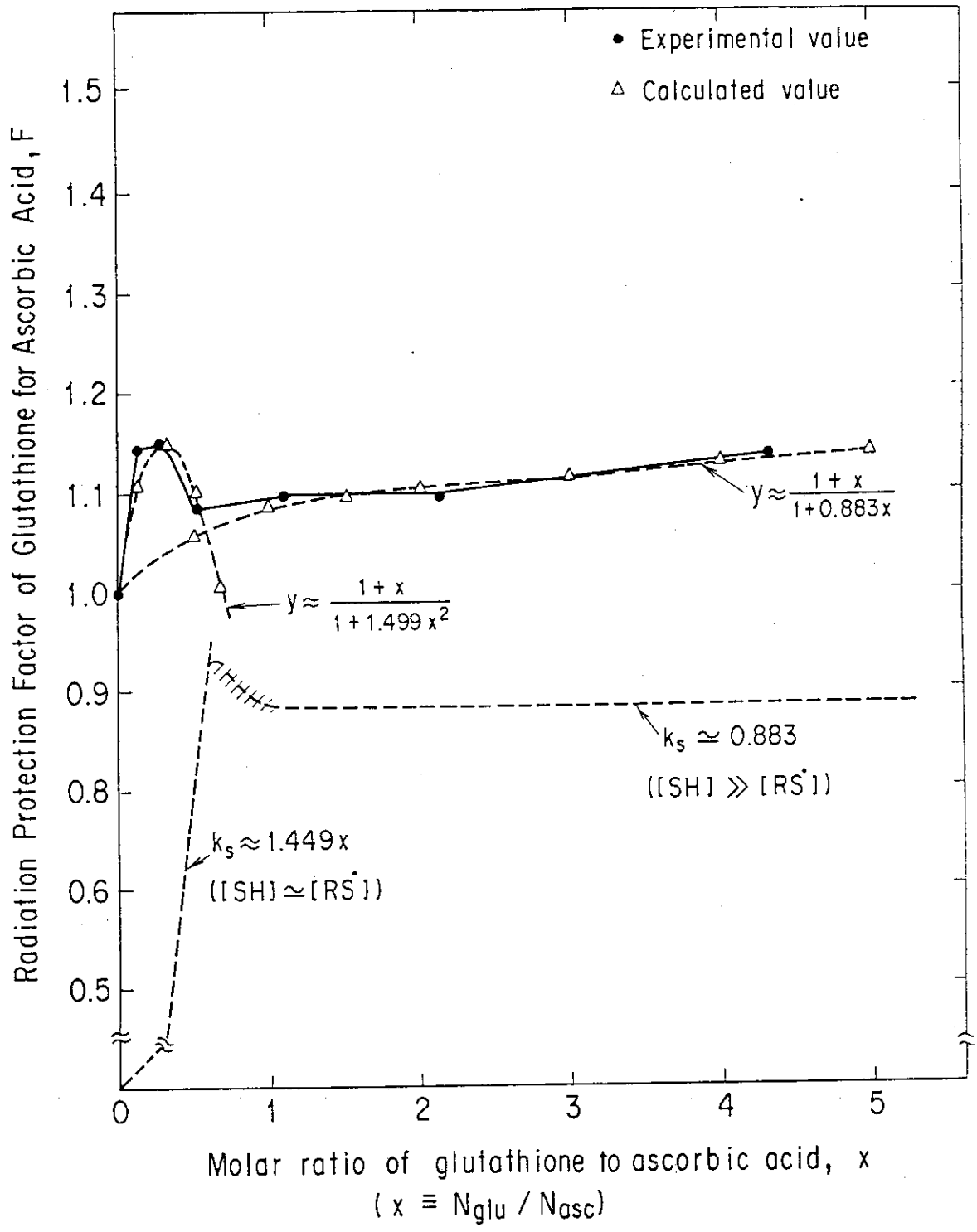
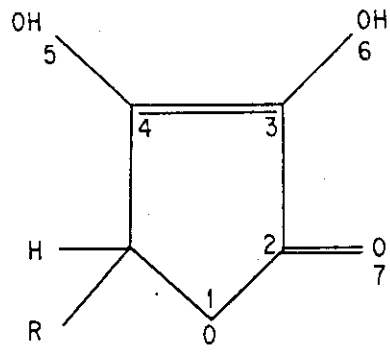


Fig. 1.6 The calculated radiation protection factor of glutathione for ascorbic acid.

Table 1.1 The reaction indices of ascorbic acid, f_r and S_r*.



Frontier electron densities :

position number (r)

	1	2	3	4	5	6	7
$f_r^{(E)}$	0.02238	0.1962	0.2600	0.1119	0.3866	0.8986	0.1243
$f_r^{(N)}$	0.04129	0.7152	0.001728	0.7185	0.2930	0.000706	0.2294
$f_r^{(R)}$	0.03183	0.4557	0.1308	0.4152	0.3398	0.4496	0.1768

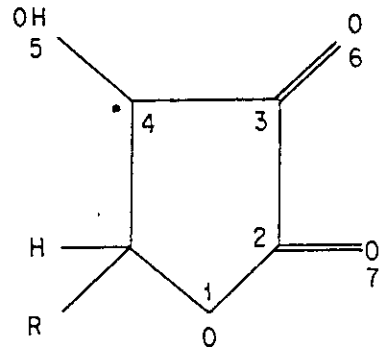
Superdelocalizabilities :

position number (r)

	1	2	3	4	5	6	7
$S_r^{(E)}$	1.0352	1.1023	1.7287	1.0418	3.0318	4.9982	1.1956
$S_r^{(N)}$	0.09083	1.7213	0.5457	1.7167	0.6169	0.05437	0.5047
$S_r^{(R)}$	0.5630	1.4118	1.1372	1.3792	1.8243	2.5262	1.1489

* HMO calculations were done using parameters suggested by T. Yonezawa, et al. (5).

Table 1.2 The reaction indices of ascorbate free radical, S_r*.

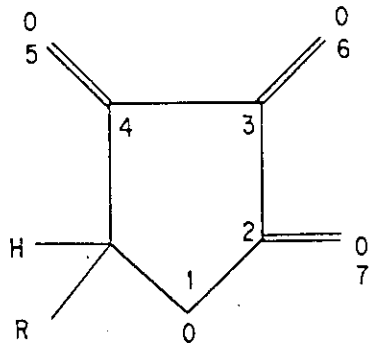


Superdelocalizabilities :

	1	2	3	4	5	6	7
$S_r^{(E)}$	0.9238	0.1934	0.2490	0.6071	0.8679	0.6480	0.5772
$S_r^{(N)}$	0.1451	2.1403	1.2726	1.8715	1.6744	0.5883	0.8061
$S_r^{(R)}$	0.5345	1.1669	0.7608	1.2393	1.2712	0.6182	0.6917

* HMO calculations were made on ascorbate radical π－anion. One electron was extracted from HOMO of the ascorbate radical π－anion.

Table 1.3 The reaction indices of dehydroascorbic acid, f_r and S_r*.



Frontier electron densities :

Position number (r)

	1	2	3	4	5	6	7
$f_r^{(E)}$	1.6950	0.0	0.0	0.0	0.0	0.0	0.3049
$f_r^{(N)}$	0.03737	0.2736	0.5599	0.2822	0.2141	0.4250	0.2076
$f_r^{(R)}$	0.8662	0.1368	0.2799	0.1411	0.1070	0.2125	0.1038

Superdelocalizabilities :

Position number (r)

	1	2	3	4	5	6	7
$S_r^{(E)}$	0.9214	0.1893	0.1817	0.1749	0.5372	0.5233	0.5633
$S_r^{(N)}$	0.1797	2.3081	1.9994	2.3684	1.0218	1.2022	0.9987
$S_r^{(R)}$	0.5505	1.2487	1.0905	1.2716	0.7795	0.8627	0.7810

* HMO calculations were done using parameters suggested by T. Yonezawa, et al. (5)

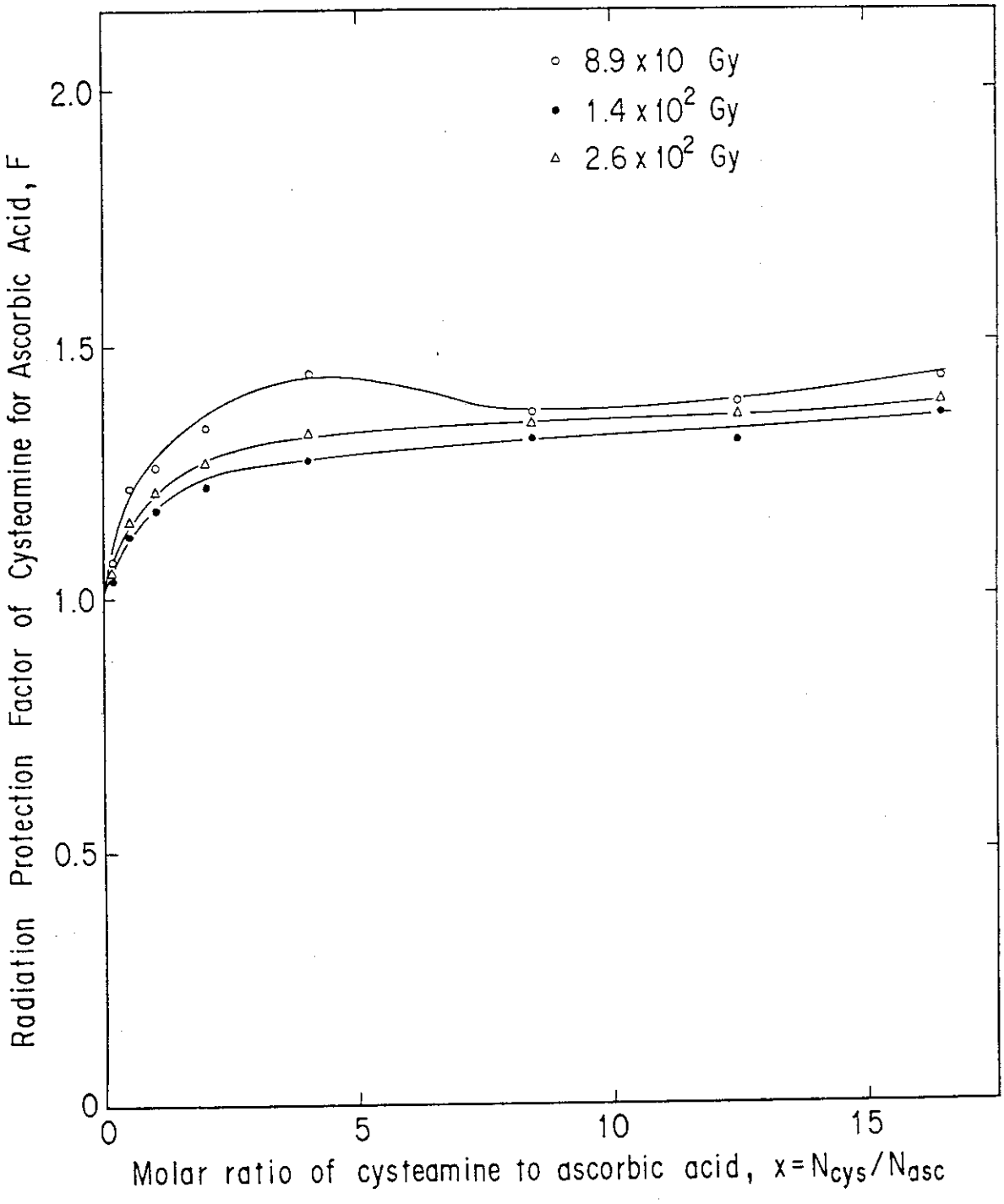


Fig. 1.7 Radiation protection factor of cysteamine for ascorbic acid in aqueous solution, as a function of molar ratio of cysteamine to ascorbic acid.

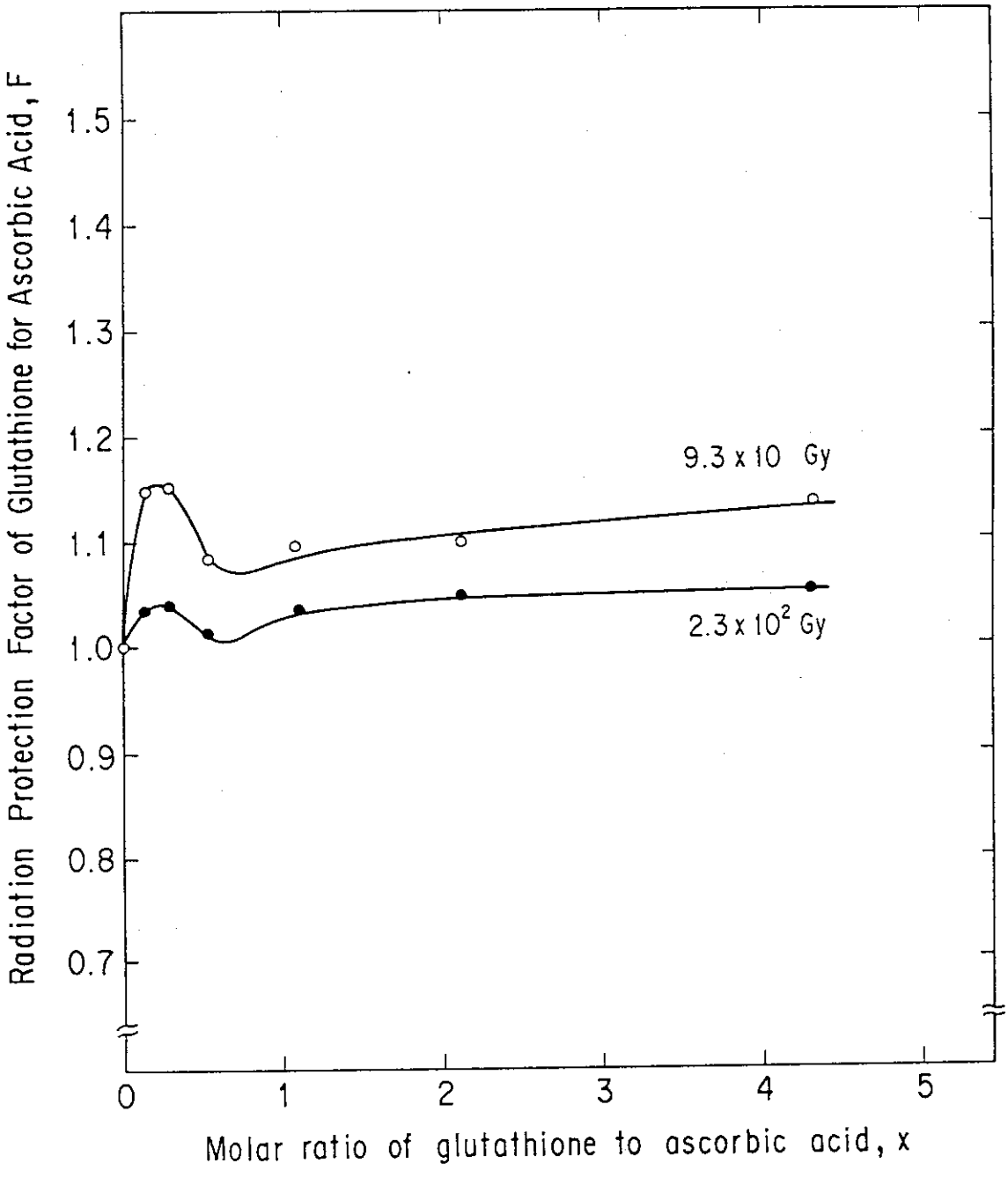


Fig. 1.8 Radiation protection factor of glutathione for ascorbic acid in aqueous solution, as a function of molar ratio of glutathione to ascorbic acid.

Fig.1.7 and 1.8 show the protection factor F of cysteamine and glutathione for ascorbic acid. The maximum value of F is about 1.45 for cysteamine and about 1.15 for glutathione at most. The protection factor F does not increased so much, even though the cysteamine or glutathione concentration increases in aqueous solution.

1.4 Conclusion.

The above analysis result leads to the following conclusion;

(1) Cysteamine or glutathione cannot protect ascorbic acid so much from the radiation damage. The protection factor F is about 1.45 for cysteamine and 1.15 for glutathione at most, even though each the chemical concentration increases.

(2) The secondary reaction takes place in irradiated solution. Cysteamine and glutathione protect ascorbic acid from the primary reaction of water radicals at first. However, cysteamine radical RS· catalizes the dissociation of hydrogenperoxide and generates another superoxide radical HO_2. The superoxide radical extracts again one electron from ascorbic acid.

The π-system of thymine is mainly broken by the additive reaction of water radicals. While, the π-system of ascorbic acid is broken, at first by the extractive reaction of water radicals, and secondly by the additive reaction of the dissolved oxygen and/or superoxide radical.

2. Autooxidation of ascorbic acid in aqueous solutions containing several kinds of antioxidants.

2.1 Experimental section.

Millimollar solutions of ascorbic acid (～20mg/l) containing cysteamine in various concentrations were stirred in the air open beekers. Ascorbic acid concetration in aqueous solution was decreased very rapidly by autooxidization. Ascorbic acid concentrations during

the autooxidation were measured by spectrophotometry. The autooxidation rate depended strongly on cysteamine concentration in water. The life time of ascorbic acid was increased very much with the cysteamine concentration. Since it was difficult to determine the whole life time of ascorbic acid accurately, the half life time of ascorbic acid were determined by using the measurment data obtained during the autooxidation.

As in the case of cysteamine, the autooxidation of ascorbic acid in aqueous solutions containing cystamine, glutathione, catalase and many kinds of spices, such as horse-radish, papain, turmeric, etc., were measured.

2.2 Results.

Fig.2.1 shows the autooxidation characteristics of ascorbic acid in aqueous solution. The region A in the figure, corresponds to the oxidation of ascorbic acid by the dissolved oxygen in water, and the region B corresponds to the oxidation by the oxygen absorbed from the air, or the surface oxidation of ascorbic acid.

Fig.2.2 shows the autooxidation curves of ascorbic acid in aqueus solutions containing cysteamine in various concentrations, as a function of time in hours. As shown in the figure, a small quantity of cysteamine prolonged very much the life time of ascorbic acid. Although the whole life time is not clear, the half life time can be determined accurately by using the autooxidation curve.

Fig.2.3 shows the autooxidation curves in the case of cystamine, which is an oxidized form of cysteamine. The half life time of ascorbic acid in the figure was almost the same as that in the absence of cystamine.

Fig.2.4 shows the autooxidation curves in the case of glutathione. The half life time of ascorbic acid in the figure was increased with increasing glutathione concentration, except the case of glutathione concentration 5mg/l. Such a characteristics of the autooxidation curve appears in ascorbic acid solution containing the other anti-

oxidants. Fig.2.5 shows the autooxidation curves in the case of horse-radish. The half life time at horse-radish concentration 10mg/l is shorter than that at the concentration 8mg/l. The half life time of ascorbic acid was increased linearlly with a dilute concentration of the other antioxidants, but was decreased and/or saturated with a dense concentration of the antioxidants.

2.3 Analysis and Discussion.

As shown in Fig.2.1, ascorbic acid concentration in aqueous solution is decreased at first exponentially with time, then decreased slowly with squear of time. The region A in the figure corresponds to the oxidation of ascorbic acid by the dessolved oxygen, and the region B corresponds to the oxidation by the oxygen absorbed from the air. If the oxidation of ascorbic acid by the dissolved oxygen is neglected, the relation of ascorbic acid concentration with time during the autooxidation holds as follows.

$$AH_2 + O_2 \underset{(\ k\ )}{\rightarrow} A\cdot H + HO_2$$

$$-\frac{d[AH_2]}{dt} = k[O_2][AH_2]$$

$$[O_2] = a\cdot t\ .$$

where, k is the reaction rate constant of oxygen with ascorbic acid. It is assumed that the oxygen concentration $[O_2]$ is linear with time. Then,

$$-\frac{d[AH_2]}{dt} \sim ak[AH_2]\cdot t\ ,$$

and, $$[AH_2] = [AH_2]_0 \exp(-akt^2/2). \qquad (2.1)$$

To explain the characteristics of Fig.2.2, it needs to introduce that the repair reaction of cysteamine for ascorbate radical, which is formed on the way of the autooxidation, takes place in the solution.

$$AH_2 + O_2 \underset{(\ k\ )}{\rightarrow} A\cdot H + HO_2$$

$$AH_2 + RS\cdot \underset{(\ k_r\ )}{\leftarrow} A\cdot H + RSH \qquad (2.2)$$

where, k_r is the repair reaction rate constant of cysteamine for the ascorbate radical. Both of ascorbic acid concentration $[AH_2]$ and cysteamine concentration [SH] are the time dependent function. The k_r is a time dependent function, also. If the k_r is assumed to be constant with time, the $[AH_2]$ can be soleved as follows.

$\overline{k_r}$ is defined as the average repair probability of cysteamine for ascorbate radical during the half-life time of autooxidation. The $\overline{k_r}$ must be averaged by the initial cysteamine concentration $[SH]_0$ and the half-life time $\Delta t=T_{1/2}$. The $\overline{k_r}$ is assumed to be constant during the half-life time $T_{1/2}$ of the autooxidation.

Then,

$$-\frac{d[AH_2]}{dt} \sim k[O_2][AH_2](1-\overline{k_r}[SH]_0) \tag{2.3}$$

and, k_r may be written as follows.

$$\overline{k_r} = \frac{\int_0^{T_{1/2}} k_r(t)[SH(t)]dt}{[SH]_0\, T_{1/2}}$$

where, $k_r(t)$ is a time dependent funtion, but $\overline{k_r}$ is a constant with time. Then, the solution of Eq(2.4) is obtained as follows.

$$[AH_2] = [AH_2]_0 \exp\left(-\frac{ak}{2}(1-\overline{k_r}[SH]_0)t^2\right) . \tag{2.4}$$

The half life time of ascorbic acid, $T_{1/2}$ in aqueous solution containing cysteamine can be written as the following equation.

$$T_{1/2} = \sqrt{\frac{2\ln 2}{ak(1-\overline{k_r}[SH]_0)}} .$$

From Eq(2.1), the half-life time, $(T_{1/2})_0$ in the absence of cysteamine can be written as folows,

$$(T_{1/2})_0 = \sqrt{\frac{2\ln 2}{ak}} .$$

Then, square of the half-life time ratio, H^2 of ascorbic acid solution with and without cysteamine,

$$H = (T_{1/2})/(T_{1/2})_0 = \frac{1}{\sqrt{1-\overline{k_r}[SH]_0}}$$

or,

$$H^2 = (T_{1/2})^2/(T_{1/2})_0^2 = \frac{1}{1 - \overline{k_r}[SH]_0}$$

$$= \frac{1}{1 - \overline{k_r}[AH_2]_0\, x} \qquad (2.5)$$

$$\sim 1 + \overline{k_r}[AH_2]_0\, x \quad , \quad \text{in a small } x.$$

where, $x = [SH]_0/[AH_2]_0$.

Fig.2.6 shows square of the half-life time ratio, H^2, as a function of molar ratio x of cysteamine to ascorbic acid. The case of cystamine is shown also in the figure. The variation of the H^2 is increased linearlly with x in the case of cysteamine, but not increased in the case of cystamine. Fig.2.7 shows the H^2 curve in the case of glutathione. A little strange characteristics of the H^2 curve can be explained as follows.

$$AH_2 + O_2 \rightarrow A\cdot H + HO_2 \ ,$$

$$AH_2 + HO_2 \xrightarrow[(\,k\,)]{} A\cdot H + H_2O_2 \ , \quad \text{the primary reaction}$$

$$AH_2 + RS\cdot \xleftarrow[(\,k_r\,)]{} A\cdot H + RSH \ , \quad \text{the repair reaction}$$

$$RS\cdot + HO_2 \ (\text{or } O_2) \rightarrow RSOOH \ (\text{or } RSOO\cdot)$$

$$RSOOH + H_2O_2 \rightarrow RSO\cdot + HO_2 + H_2O \ ,$$

$$AH_2 + HO_2 \rightarrow A\cdot H + H_2O_2 \ , \qquad (2.6)$$

$$AH_2 + RSO\cdot \xrightarrow[(\,k_s\,)]{} A\cdot H + RSOH \ , \quad \text{the secondary reaction.}^{(6)}$$

where, k_s is the secondary reaction (or reoxidation) rate constant of glutathione radical, $RS\cdot$ with ascorbic acid through reaction(2.6). To explain the characteristics of the H_2 curve in the case of glutathione, it needs to introduce another parameter $\overline{k_s}$. The $\overline{k_s}$ is defined as the reoxidation (or the secondary reaction) probability of gultathione radical $RS\cdot$ with ascorbic acid, which must be averaged by initial ascorbic acid concentration $[AH_2]_0$ and the half-life time $\Delta t = T_{1/2}$. The $\overline{k_s}$ is assumed to be constant during the half-life time, as well as the repair probability $\overline{k_r}$.

According to the assumptions, the following equation holds.

$$-\frac{d[AH_2]}{dt} \sim k(1-\overline{k_r}[SH]_0)[O_2][AH_2] + k\cdot\overline{k_r}\,\overline{k_s}[SH]_0[O_2][AH_2]^2 \qquad (2.7)$$

where, the k_s is represented as follows,

$$\overline{k_s} = \frac{\int_0^{T_{1/2}} k_s(t)[AH_2(t)]dt}{[AH_2]_0 T_{1/2}}$$

The solution of Eq(2.8) is obtained in the following relation.

$$[AH_2] = \frac{1}{\left\{\frac{1}{[AH_2]_0} + \left(\frac{\overline{k_r}\,\overline{k_s}[SH]_0}{1-\overline{k_r}[SH]_0}\right)\right\}\exp(k(1-\overline{k_r}[SH]_0)at^2/2) - \left(\frac{\overline{k_s}\,\overline{k_r}[SH]_0}{1-\overline{k_r}[SH]_0}\right)}.$$

Then,

$$T_{1/2} = \sqrt{\frac{2}{ak(1-\overline{k_r}[SH]_0)} \cdot \ln 2 \; \frac{1-\overline{k_r}[SH]_0(1-\overline{k_s}[AH_2]_0/2)}{1-\overline{k_r}[SH]_0(1-\overline{k_s}[AH_2]_0)}},$$

$$H^2 = (T_{1/2})^2/(T_{1/2})_0^2$$

$$= \frac{1+\{\ln(1-\overline{k_r}[AH_2]_0(1-\overline{k_s}[AH_2]_0/2)x) - \ln(1-\overline{k_r}[AH_2]_0(1-\overline{k_s}[AH_2]_0)x)\}/2\ln 2}{(1-\overline{k_r}[AH_2]_0 x)} \qquad (2.8)$$

From solution(2.8),

$$H^2 = \frac{1}{1-\overline{k_r}[AH_2]_0 x}, \quad \text{in a small } x \qquad (2.9)$$

and,

$$\lim_{x \to 1/\overline{k_r}[AH_2]_0} H^2 = \frac{1}{\ln 2}\,\frac{1}{\overline{k_s}[AH_2]_0}, \quad \text{in a large } x. \qquad (2.10)$$

As shown in Fig.2.7, the H^2 curve in a small x is equivalent to solution (2.9), and the H^2 curve in a large x corresponds to solution (2.10) in a large x.

From the variation of the H^2 curves, the repair probability $\overline{k_r}$ and the reoxidation probability $\overline{k_s}$ can be estimated in table 2.1 and 2.2, respectively. Table 2.1 means that cysteamine repairs 99.4% of ascorbate radicals on the way of autooxidation at x=0.31. Table 2.2 means that glutathione repairs 98.6% of the ascorbate radicals at x=0.01. Glutathione repairs almost 100% of the ascorbate radicals at x=0.103. The secondary reaction, however, reoxidizes about 2.3% of initial ascorbic acid concentration during the half life time.

Eq(2.10) is equivalent to Eq(1.10) in chapter 1. The autooxidation characteristics of ascorbic acid solution containing cysteamine and/or glutathione corresponds to the radiation protective effects of the

chemicals on ascorbic acid. Irradiation just accelerates the oxidation of ascorbic acid.

Fig.2.8 shows the presumable autooxidation process of ascorbic acid in aqueous solution containing sulfhydryl compounds. Dehydroascorbic acid accumulated couldn't be restored to the original ascorbic acid by adding cysteamine and/or glutathione. While sulfhydryl compound are able to repair ascorbate radical on the way of autooxidation, and prolonged the life time of ascorbic acid. Ascorbate radical may have two states on the way of autooxidation; the first state in which one electron remains still at $(OH)_6$ position of ascorbic acid, and the second state in which C-O· radical at the position is changed to C=O bond through the enol-keto transition. It is considered that the enol form of ascorbate radical (shown in Fig.2.8,(b) A·H) can be repaired by sulfhydryl group to original ascorbic acid, but the keto form of ascorbate radical (shown in the Fig.2.8,(c) A·H), cannot be repaired by the sulfhydryl group.

Fig.2.9 shows the autooxidation curves of ascorbic acid in aqueous solutions containing several kinds of spices, such as cinnamon, laurel, clove, horse-radish, garlic, mustard, paprika, and so on. As shown in the figures, some of these spices may contain a plenty of sulfhydryl compounds and/or the other antioxidants. Fig.2.10 ～2.13 show the autooxidation curves of ascorbic acid solutions containing papain, turmeric, redpepper and catalase (from Bovine Liver) in various concentrations. As shown in Fig.2.13, some of the autooxidation curves in the case of catalase exceed unity with time. The photopeak wave length shifts from 258nm to 278nm, and the absorptivity was increased very much. Ascorbic acid reduces catalase right after mixing them. It seems that Fe(Ⅲ) in catalase is changed to Fe(Ⅱ). The absorption spectra in ascorbic acid solution containing catalase are for the Fe(Ⅱ) photoabsorption. The autooxidation rate of Fe(Ⅱ) itself is higher than that of ascorbic acid.

Fig.2.14 summarizs square of the half-life time ratio H^2 of ascorbic acid in aqueous solutions containing several kinds of antioxidant, as

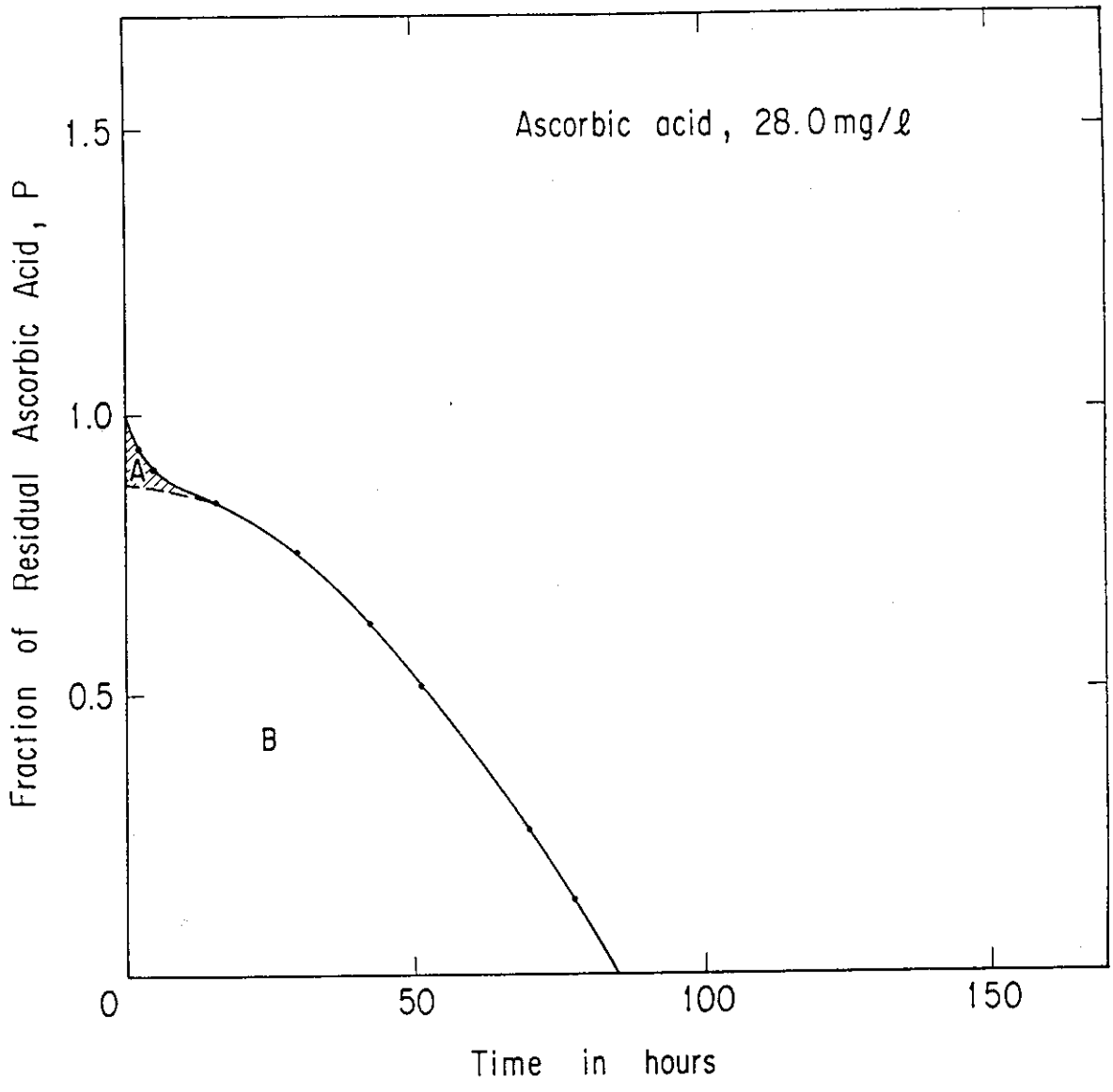


Fig. 2.1 Autooxidation of ascorbic acid in aqueous solution which is stirred in air open beeker, as a function of time in hours.

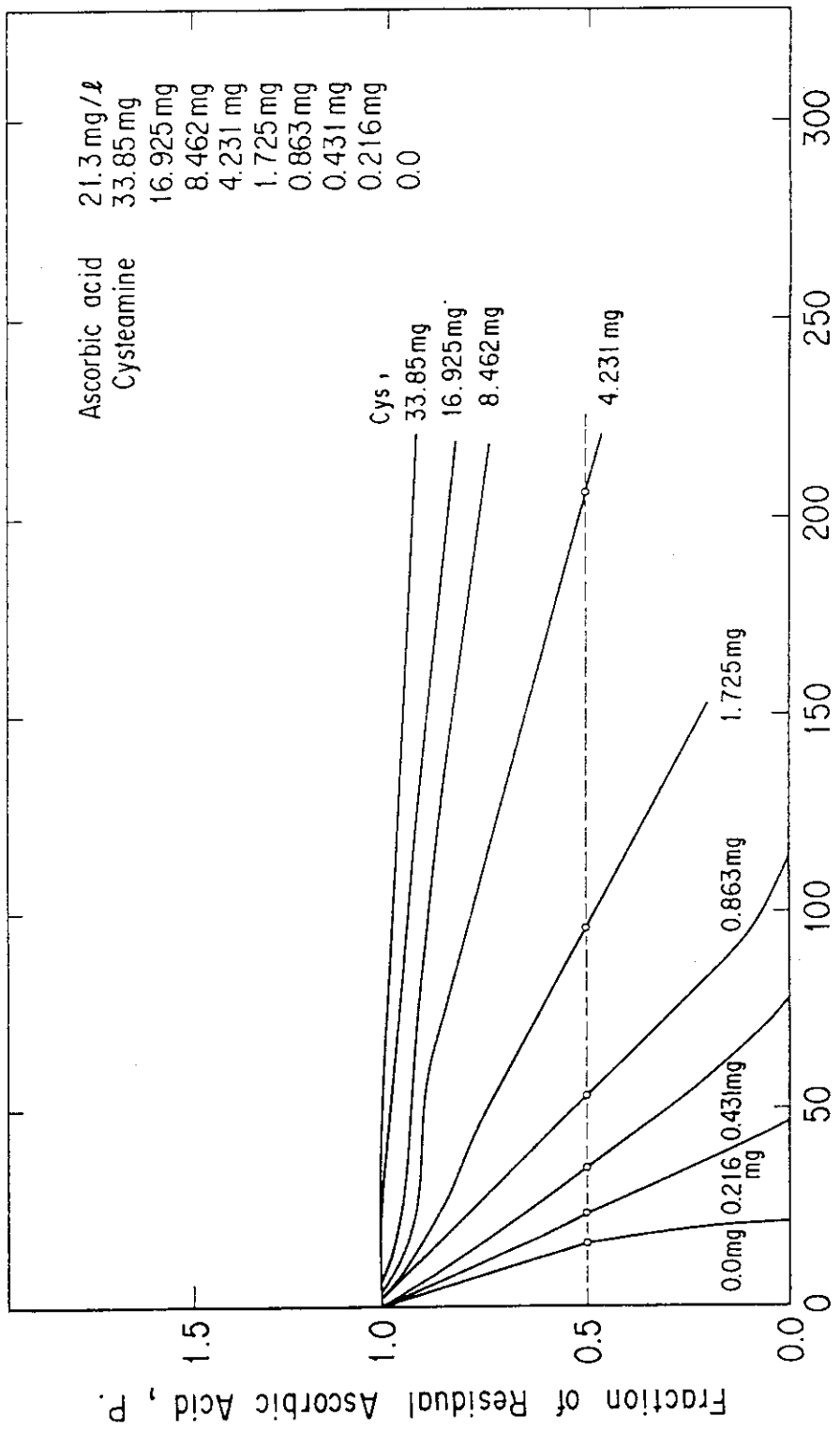


Fig. 2.2 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing cysteamine, as a function of auto-oxidation time.

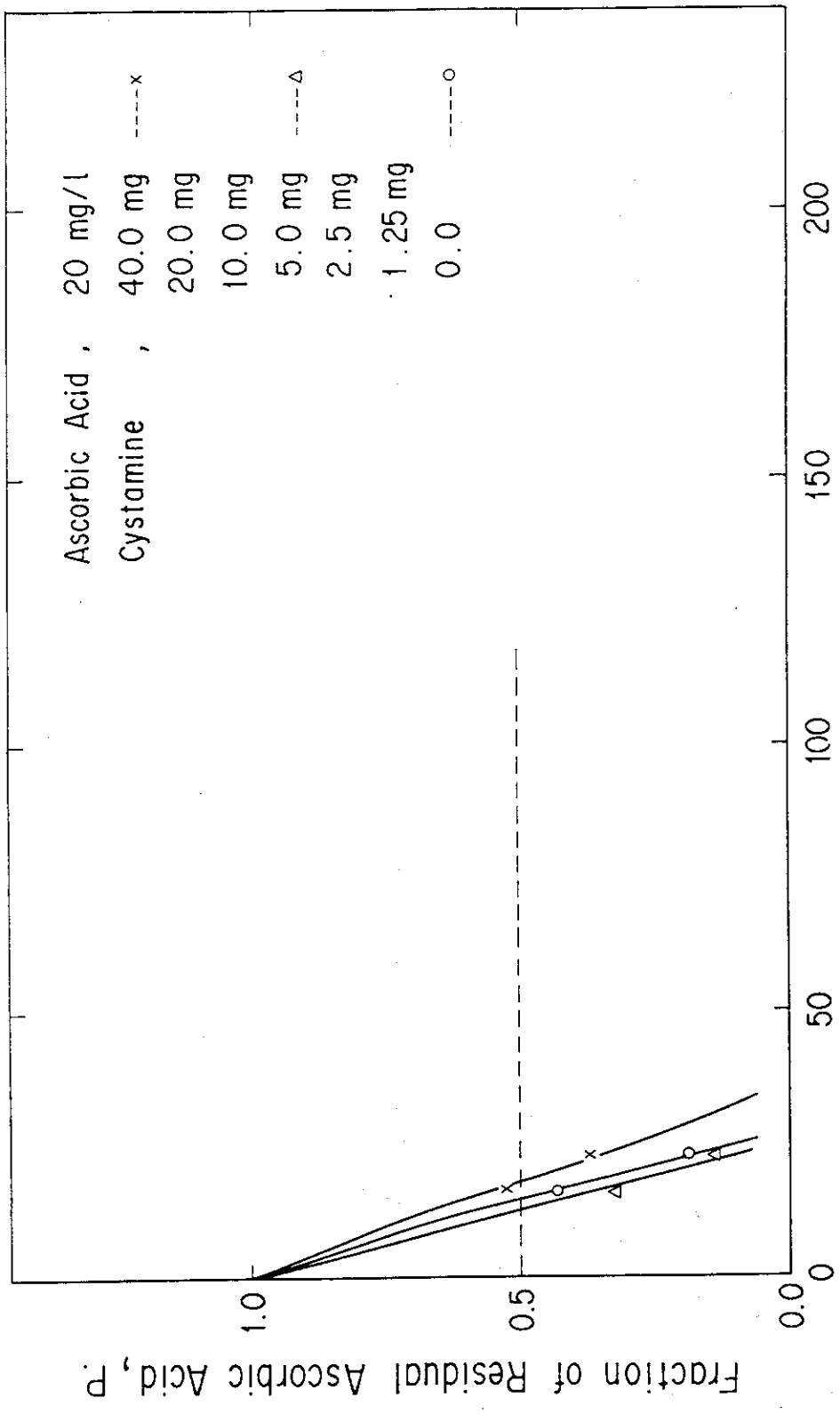


Fig. 2.3 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing cystamine (dihydrochloride), as a function of auto-oxidation time.

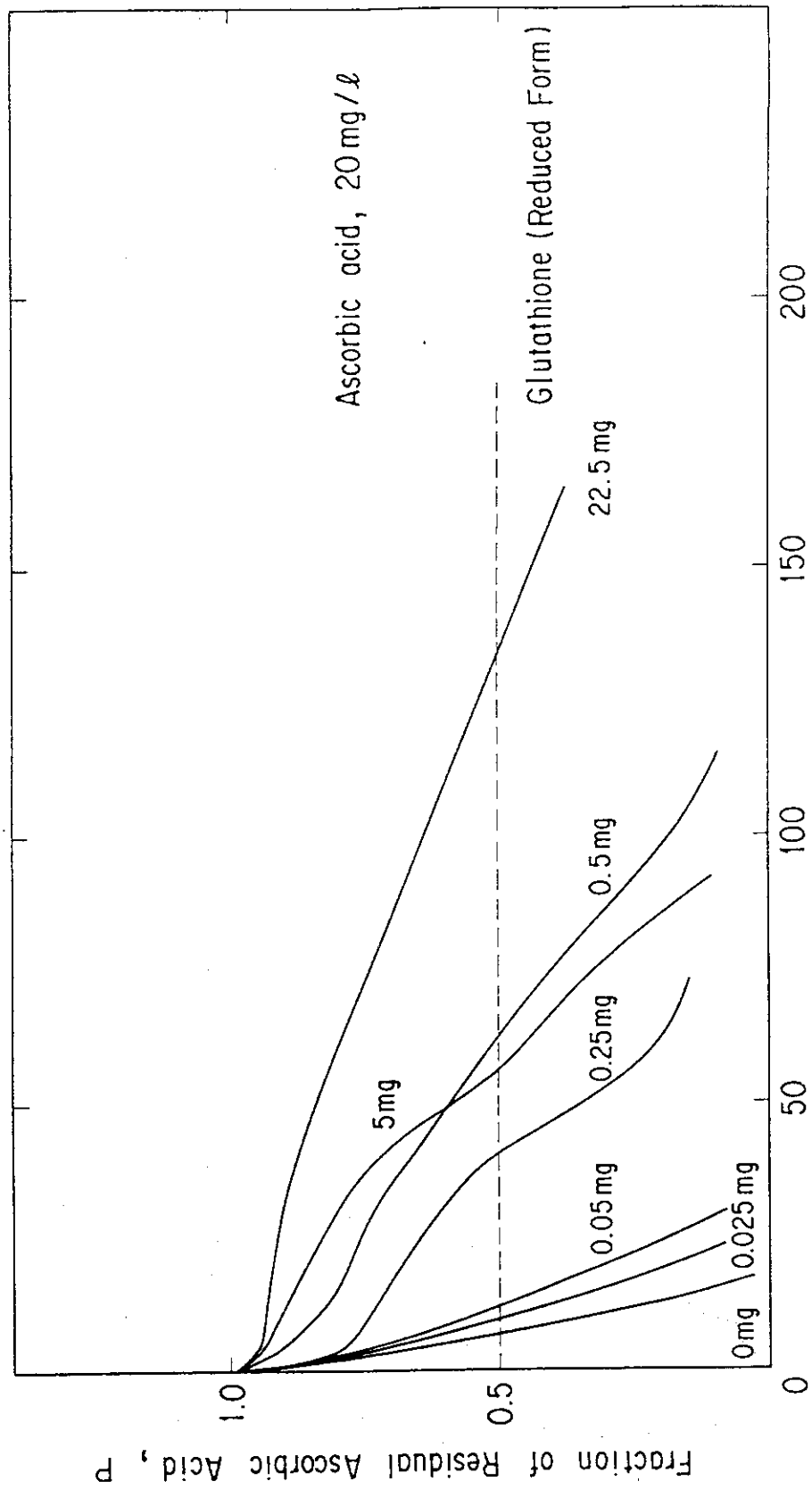


Fig. 2.4 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing glutathione (Reduced Form) as a function of time in hours.

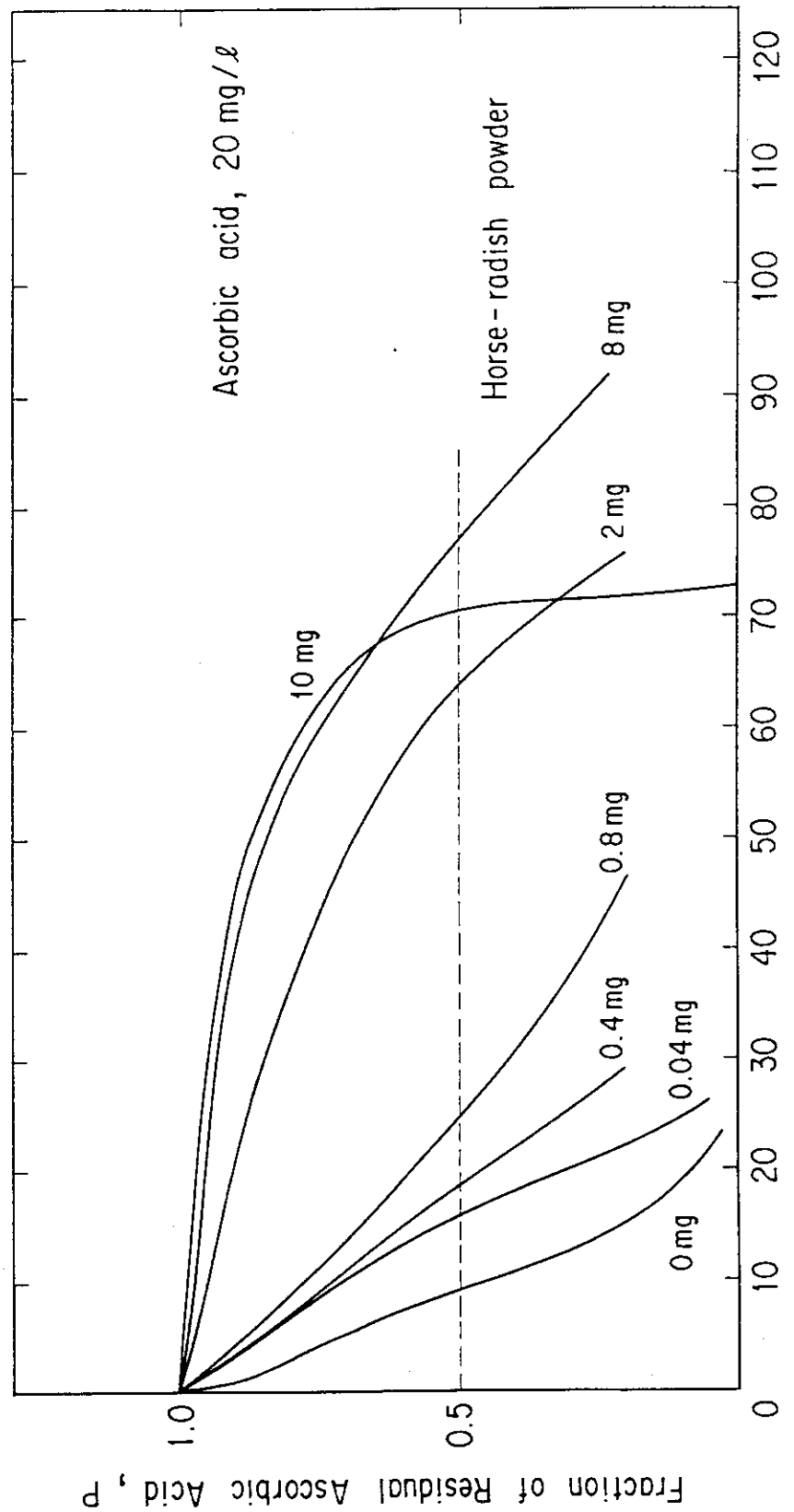


Fig. 2.5 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing horse-radish powder as a function of time in hours.

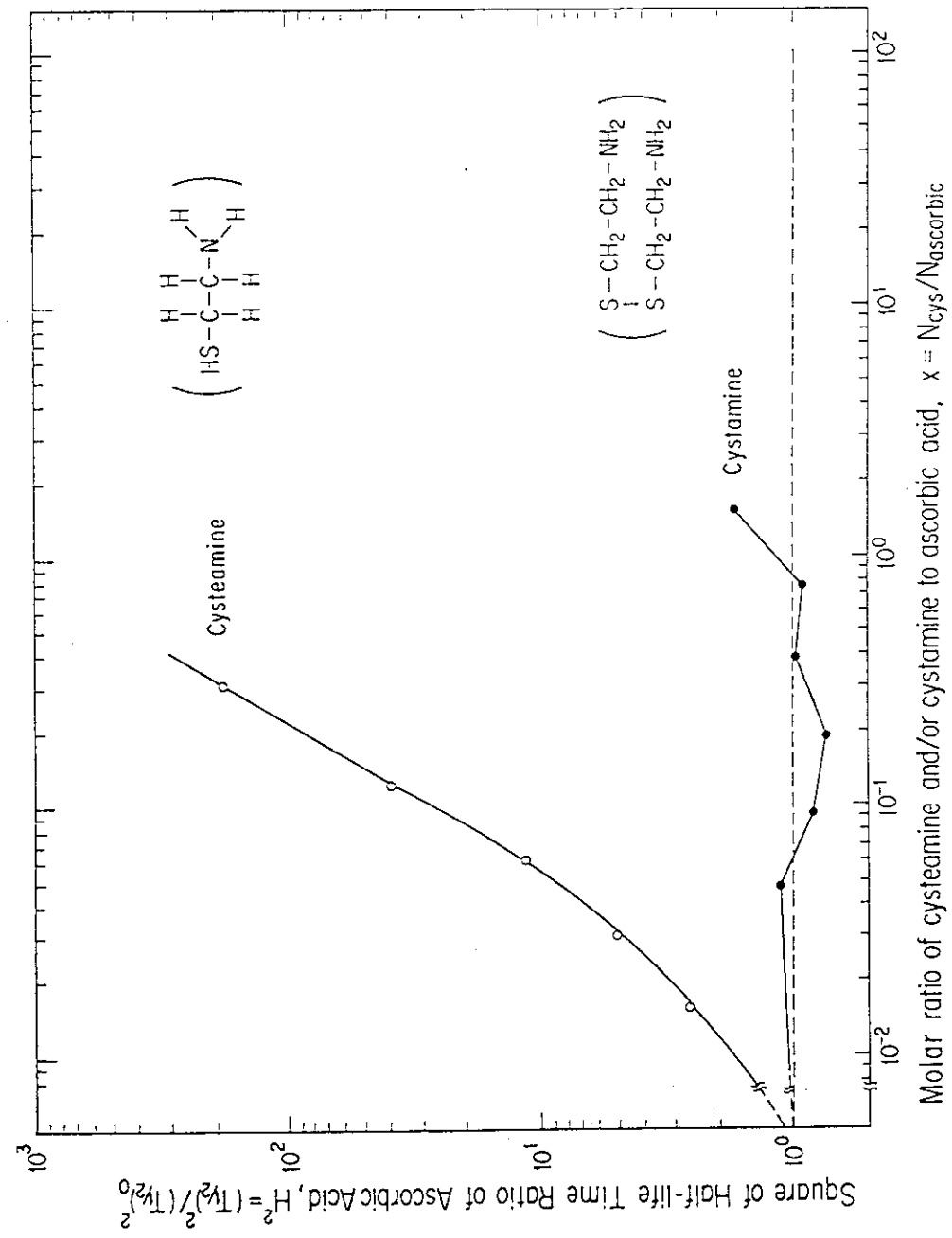


Fig. 2.6 Square of half-life time ratio of ascorbic acid in aqueous solutions containing cysteamine and/or cystamine, as a function of molar ratio of cysteamine and/or cystamine to ascorbic acid.

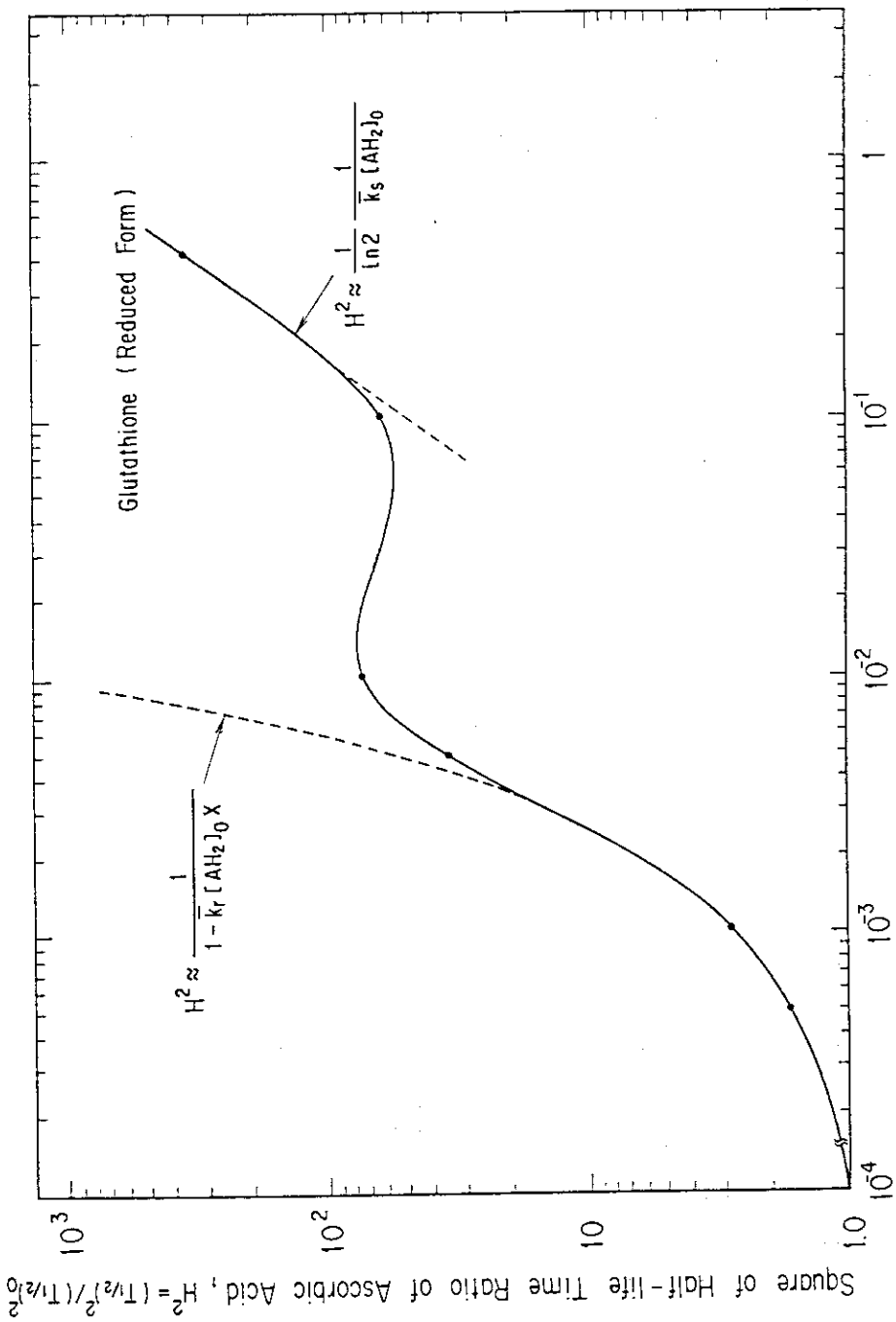


Fig. 2.7 Square of half-life time ratio of ascorbic acid in aqueous solutions containing glutathione as a function of molar ratio of glutathione to ascorbic acid.

Table 2.1 Repair probability of cysteamine for ascorbic acid during auto-oxidation in aqueous solution.

Concentration ratio $x = [SH]_0 / [AH_2]_0$	Square of Half-life time ratio $H^2 = (T_{1/2})^2 / (T_{1/2})_0^2$	Rapair probability $\overline{k_r}\ [SH]_0$
0.016	2.5	0.600
0.031	4.9	0.796
0.061	11.5	0.913
0.125	38.0	0.973
0.310	180	0.994

Table 2.2 Repair and re-oxidation probabilities of glutathione for ascorbic acid during auto-oxidation in aqueous solution.

Concentration ratio $x = [SH]_0 / [AH_2]_0$	Square of Half-life time ratio $H^2 = (T_{1/2})^2 / (T_{1/2})_0^2$	Repair probability $\overline{k_r}\ [SH]_0$	Re-oxidation probability $\overline{k_s}\ [AH_2]_0$
0.0006	1.69	0.408	
0.001	3.06	0.6732	
0.006	34.81	0.9712	
0.01	73.96	0.9864	
0.103	62.4		2.312×10^{-2}
0.451	364.8		3.954×10^{-3}

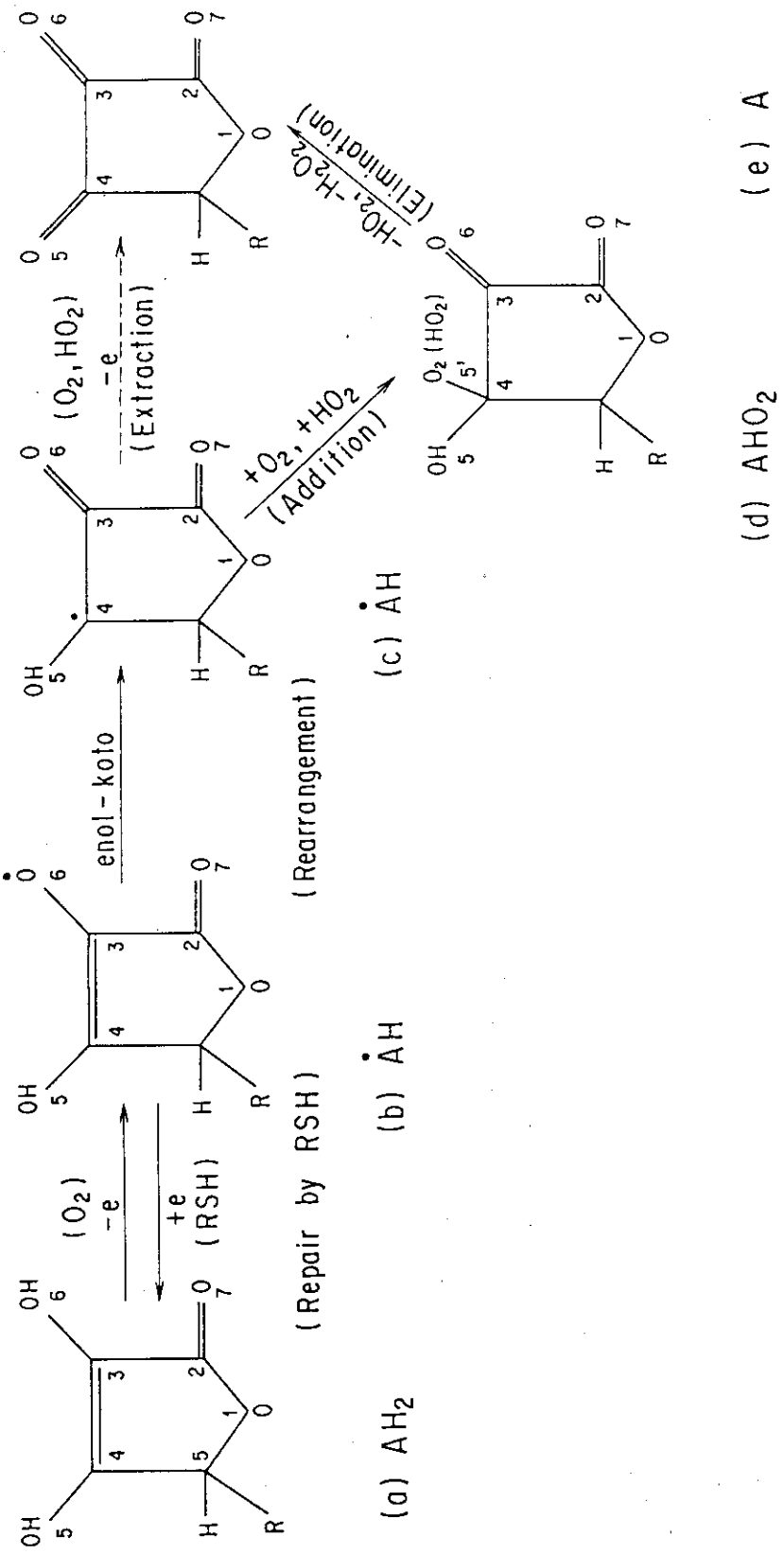


Fig. 2.8 Repair and rearrangment of ascorbate radical on the way of ascorbic acid's autooxidation in aqueous solution containing sulfhydryl compound.

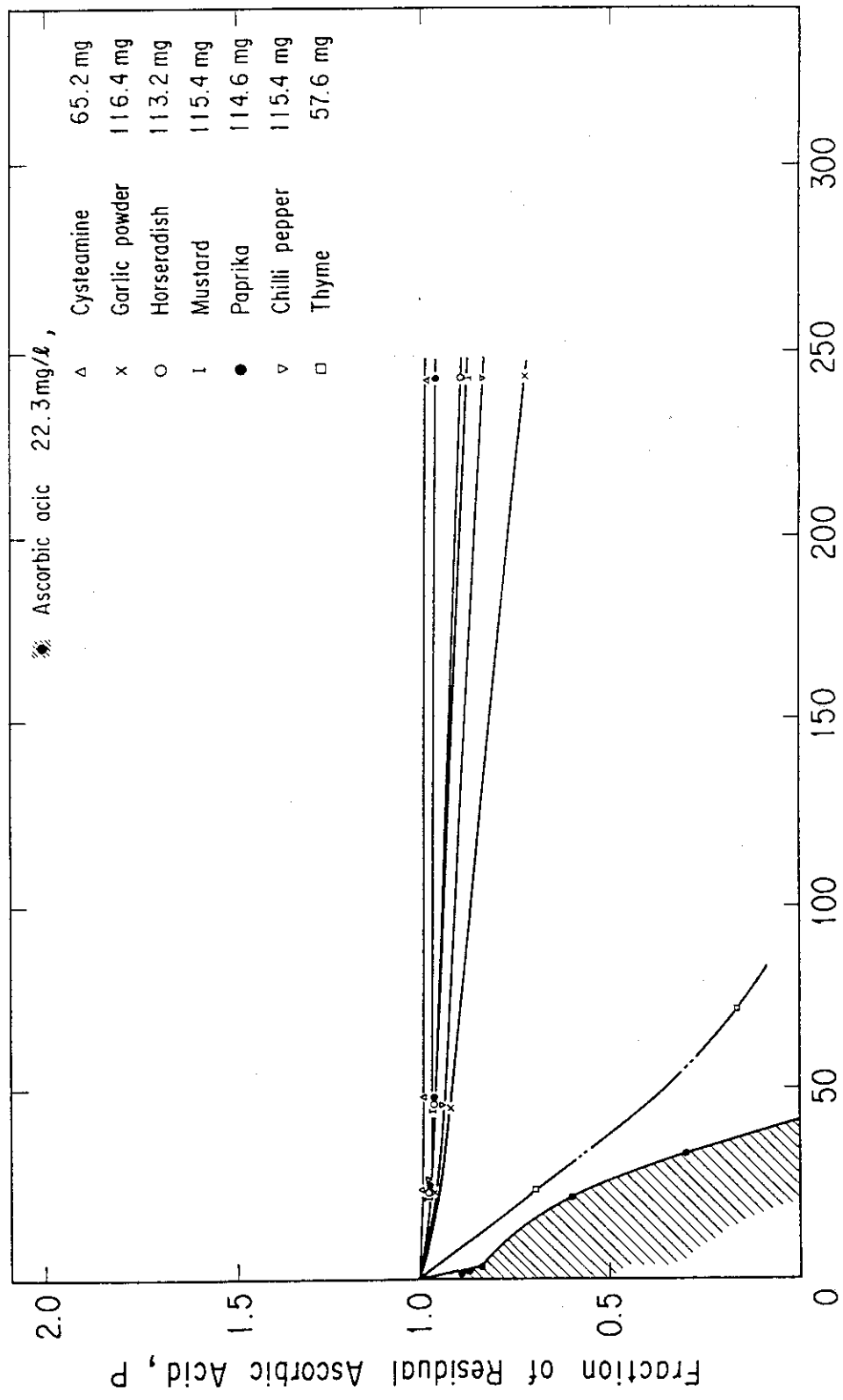


Fig. 2.9 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing several kinds of spices as a function of time in hours, during auto-oxidation.

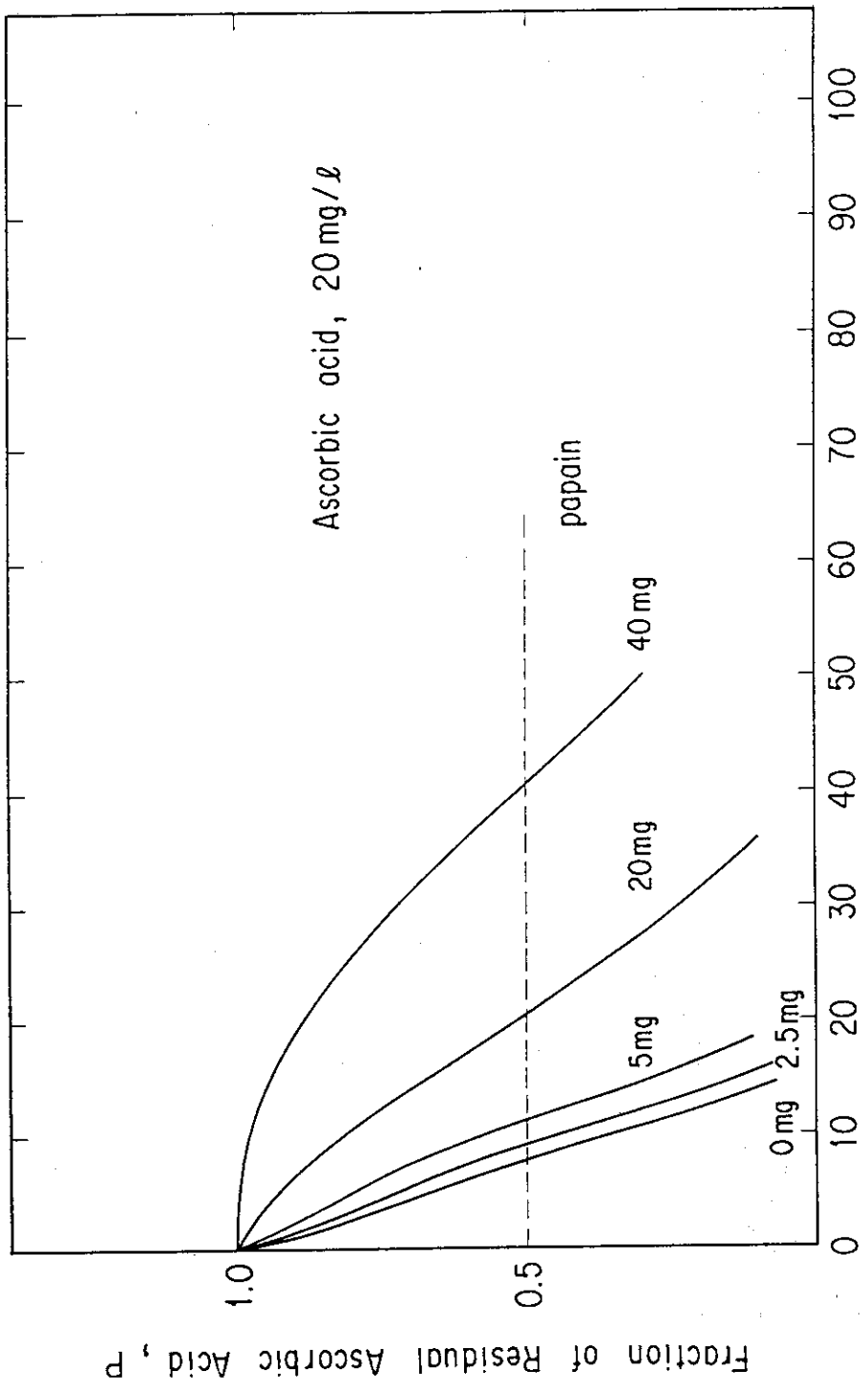


Fig. 2.10 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing papain as a function of time in hours.

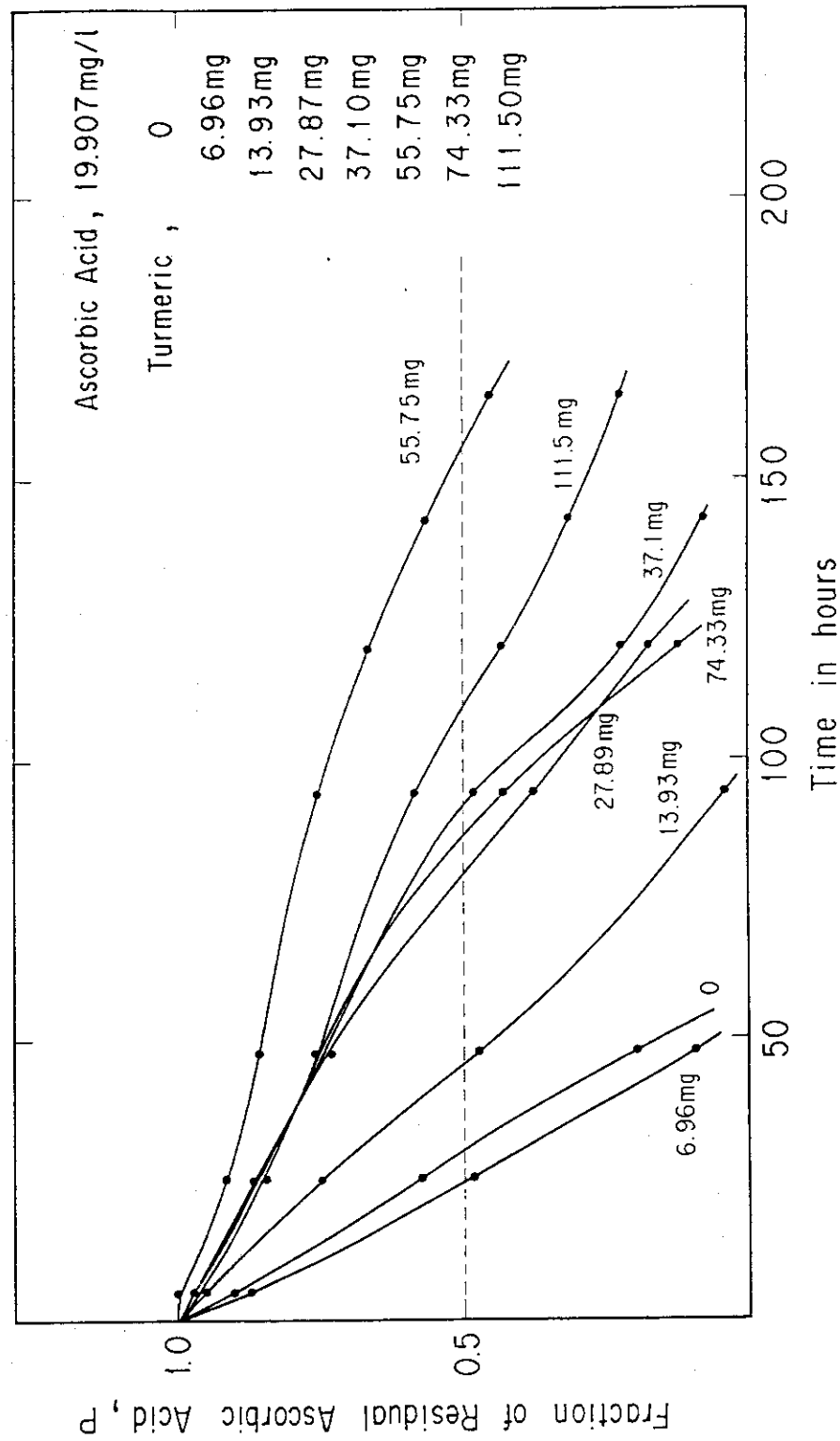


Fig. 2.11 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing turmeric, as a function of time in hours.

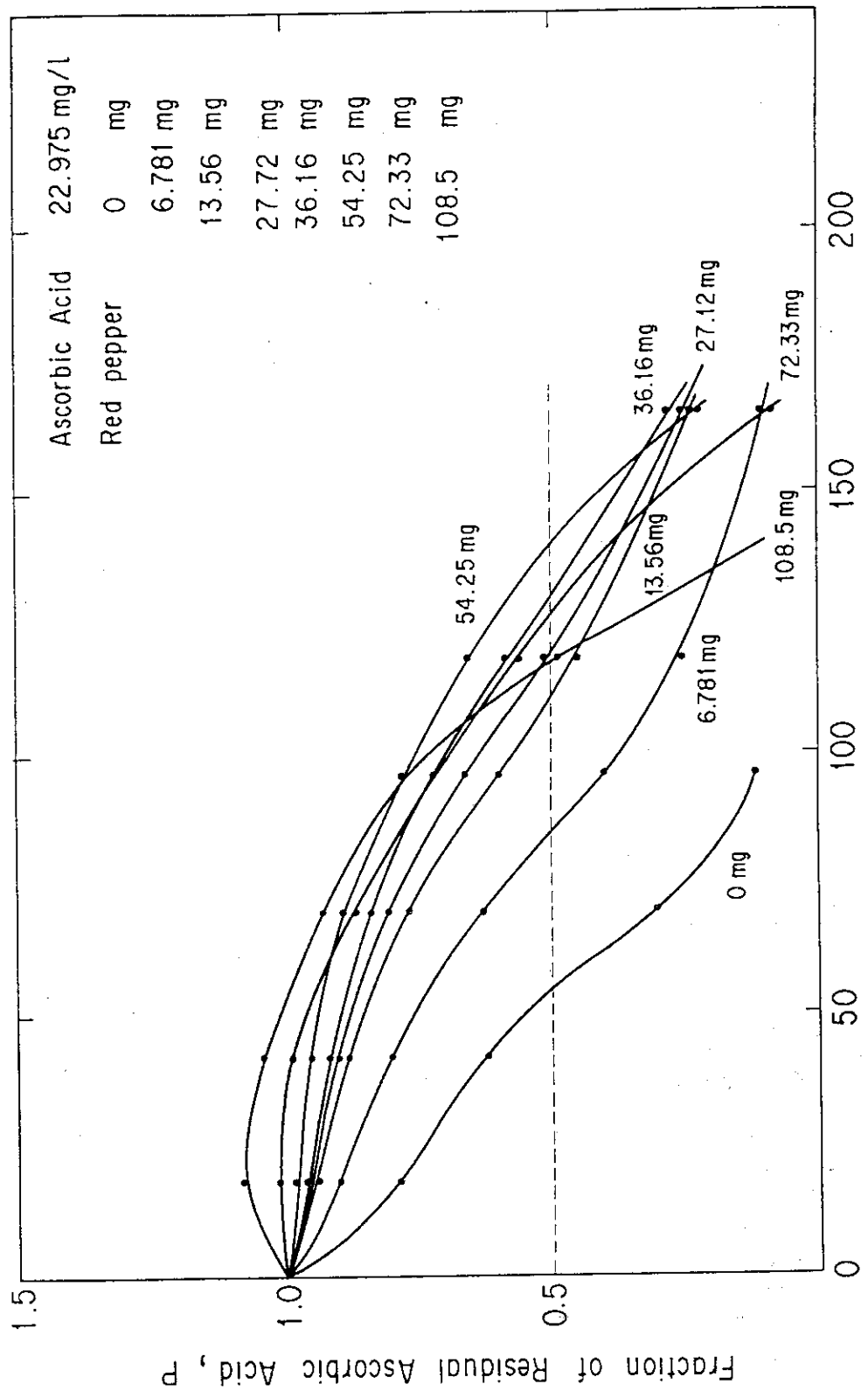


Fig. 2.12 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing red pepper, as a function of time in hours.

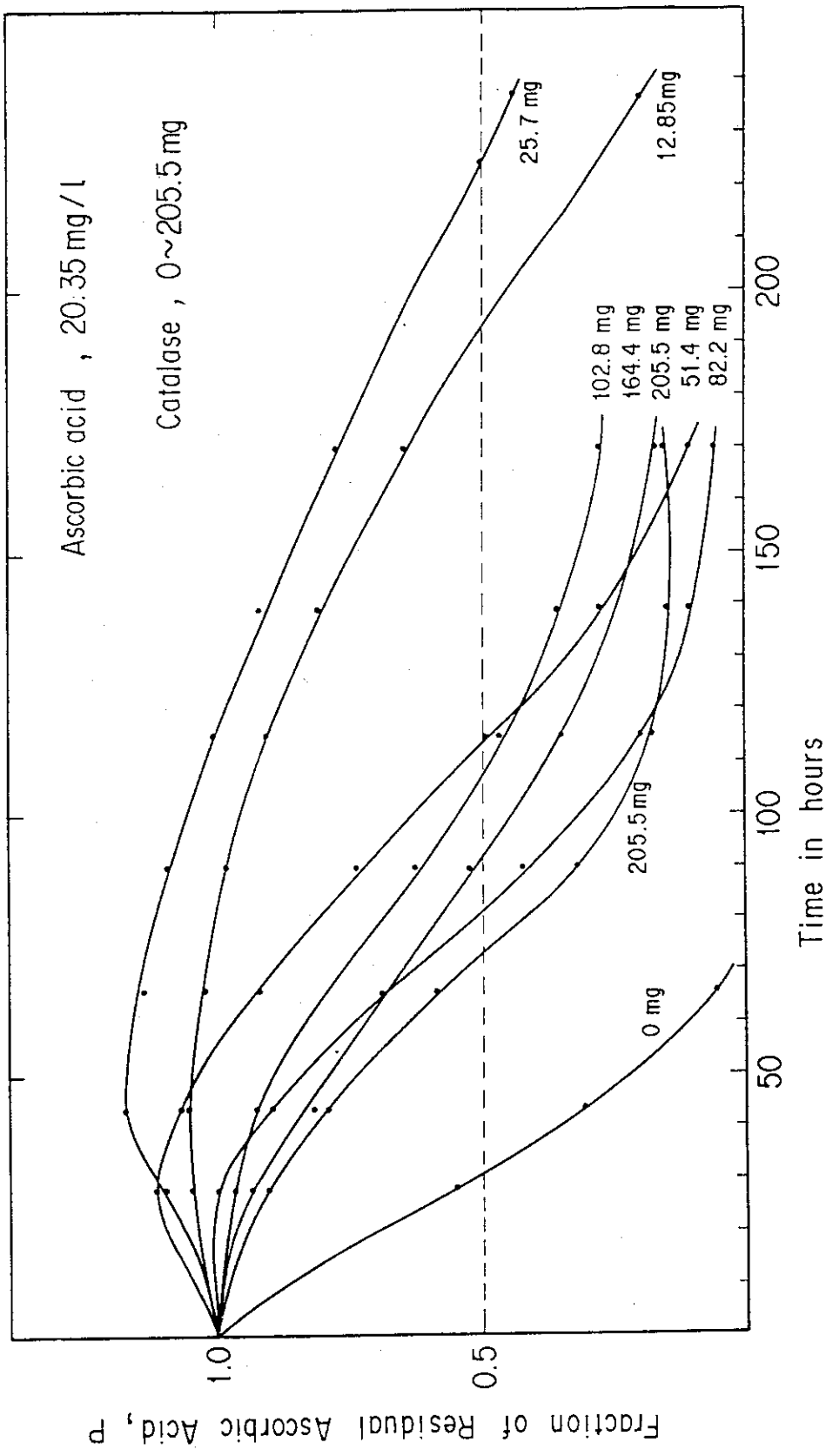


Fig. 2.13 Fraction of residual to initial concentration of ascorbic acid in aqueous solutions containing catalase (from Bovine Liver), as a function of auto-oxidation time.

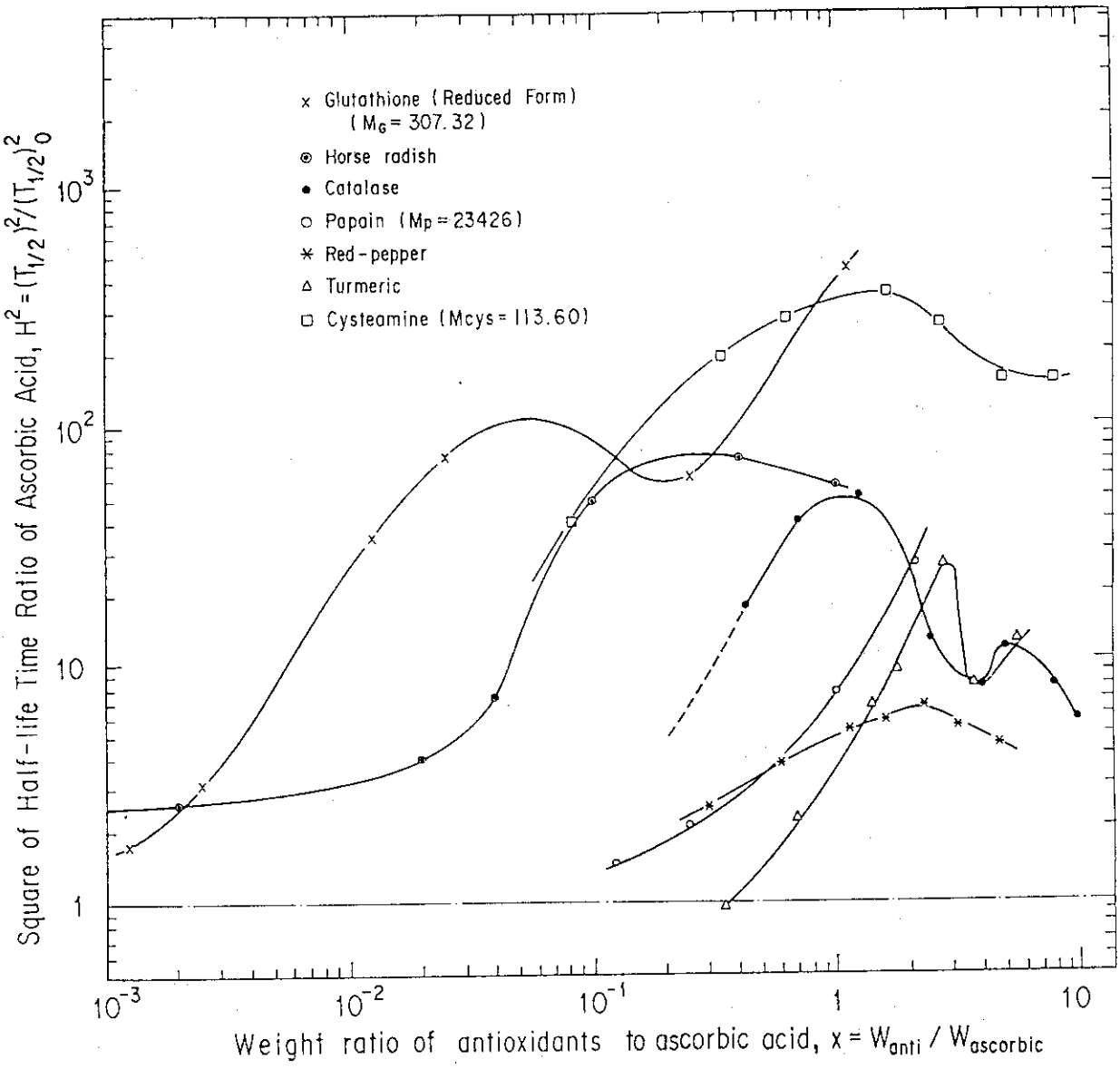


Fig. 2.14 Square of half-life time ratio of ascorbic acid in aqueous solutions containing several kinds of antioxidants as a function of weight ratio of antioxidants to ascorbic acid.

a function of weight ratio x_w of ascorbic acid to the antioxidants. As shown in the figure, horse radish and catalase are comparable to cysteamine and glutathione as an antioxidant for ascorbic acid. Turmeric and papain are a very good agent to protect ascorbic acid from autooxidation, also. Redpepper has a little antioxidizing property for ascorbic acid, and may contain some different kinds of antioxidant except sulfhydryl compounds.

2.4 Conclusion.

The above analysis and discussion leads to the following conclusion;

(1)The half life time of ascorbic acid autooxidation in aqueous solution is increased with a dilute sulfhydryl concentration. The half life time, however, is decreased or saturated with a dense sulfhydryl concentration.

(2)The half life time is increased by the repair reaction of sulfhydryl group for ascorbate radical which is formed during autooxidation of ascorbic acid.

(3)The half life time is saturated in a dense sulfhydryl solution. The secondary reaction of sulfhydryl radical RS· and hydrogenperoxide accumlated in water takes place in the dense sulfhydryl concentration.

(4)The autooxidation characteristics of ascorbic acid in aqueous solution containing sulfhydryl compounds is very simillar with the radiation protective effects of sulfhydryl compound on ascorbic acid.

(5)There are many spices which contain a plenty of antioxidants, such as horse-radish, mustard, cinnamon, garlic, laurel, papain, turmeric, etc.. The functions of these spices as an antioxidant can be estimated by measuring the autooxidation characteristics of ascorbic acid in aqueous solution containing these spices.

Acknowledgement.

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Reference.

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