STUDIES ON GLUTATHIONE

by

RUTH D. PETERSON

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INTRODUCTION

A constituent of living tissue possessing reducing power due to labile hydrogen long has been known to exist. In 1888 de Rey Pailhade[1, 2] showed that yeast cells and aqueous extracts of yeast are capable of reducing sulfur to hydrogen sulfide. He considered some hypothetical reducing substance present in yeast cells and extracts to be responsible for this production of hydrogen sulfide from sulfur and called it "philothion". In the succeeding years he published a long series of communications concerning its distribution and properties. He found that many animal tissues show the existence of labile hydrogen which he maintained to be of importance in the respiratory functions of living cells. His views as to the probable nature of this substance changed from time to time. In his latest papers he referred to it as the hydride of a protein ("hydrure d'albumine") and accepted the view of Heffter that labile hydrogen exists in tissues in the form of sulfhydryl groups, —SH.

Mörner[3] in 1901 used the nitroprusside reaction for the identification of cysteine. Later Gola[7] used this reaction to demonstrate the presence of a substance containing —SH groups in proliferating plant tissue. Buffa[7] showed that certain animal tissues give the same color reaction.

In 1908 Heffter[4] tested a great number of tissues and tissue extracts with the nitroprusside test and in many cases obtained positive results. Arnold[5, 6] in 1911 showed that under proper conditions the reaction is given by practically all organized animal tissues. A little later he came to the conclusion that cysteine is the responsible substance since protein-free extracts gave a strong nitroprusside reaction. He considered the evidence to indicate cysteine to be a primary cell constituent even though he did not isolate it.

Heffter was the first to consider that the labile hydrogen from the —SH group, whatever its associations, is responsible for the reducing properties of protoplasm and concerned with oxidations in the cell. He suggested that hydrogen peroxide might arise during the autoxidation of the sulfhydryl group and the peroxide oxygen be transferred to other substances in the cell. He felt that the spontaneous oxidation to —S—S— groups must be reversible thus permitting the continuous action of —SH groups in cell oxidations. He cited the fact that cystine can be reduced to cysteine by sodium sulfite, and suggested that some substance in the cell might act as an acceptor for the oxygen of the water molecule, the hydrogen then reducing the disulfide to sulfhydryl.

No experimental proof was given of the existence in the cell of free cysteine or any other compound containing an —SH group responsible for the nitroprusside reaction until in 1921 Hopkins[7] described the isolation from yeast, mammalian liver, and muscle of such a substance, and conducted an extensive study of its constitution and properties.

He found the compound to be a dipeptide containing glutamic acid and cysteine and named it accordingly glutathione. Its reactions fulfilled the requirements for the hypothetical substance previously described.

Hopkins first extracted glutathione from baker's yeast by boiling with water. He then carried out a series of precipitations and purifications with lead acetate, uranium acetate, mercuric sulfate, copper hydroxide and phosphotungstic acid. By a slightly modified extraction he also isolated it from mammalian liver and muscle.

According to Hopkins' analysis the substance contained glutamic acid and cysteine both of which he identified. His values for the percentages of carbon, hydrogen, sulfur and nitrogen checked with the theoretical for a dipeptide consisting of glutamic acid and cysteine. He was not able to establish the exact linkage between the two amino acids.

Hopkins' report on glutathione stimulated much interest in the substance because of its possible relation to biological oxidation-reduction systems. In the years that followed Hopkins and associates[8, 9, 10] published the results of investigations on the physiological relations of glutathione. They showed that when tissue was washed until it lost its ability to reduce methylene blue the addition of glutathione, in either reduced or oxidized form, restored its reducing power. Heating the tissue to 100° C did not affect these relations. Hopkins considered that glutathione forms a peroxide which may function as an oxidizing agent toward fatty acids and proteins.

Dixon and Tunnicliffe[11] confirmed the reducing action of glutathione and considered this action to be catalyzed by the oxidized —S—S— form. Harrison[12], Dixon and Quastel[13], and Holden[14, 15] demonstrated the activity of this oxidation-reduction system in biological materials. Harrison[16] found that the rate of oxygen uptake by glutathione was greatly reduced by removal of all traces of iron and that the inhibition by hydrogen cyanide was due to a cyanide complex with iron. The iron of hematin was capable of catalyzing the reaction.

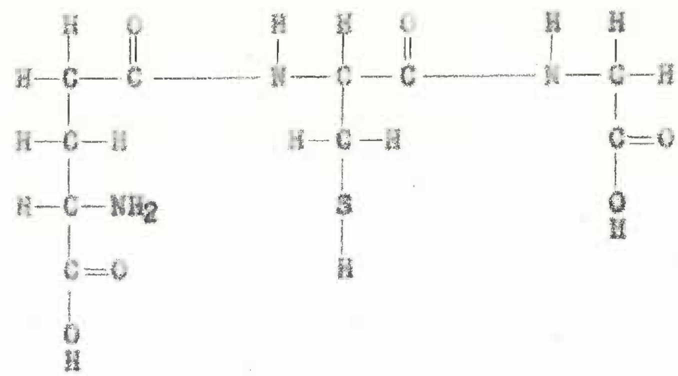
Holden[17] tested sheep blood and reported glutathione to be concentrated in the corpuscles with no trace present in the plasma.

Tunnicliffe[18] and Murray[19] worked out methods for the quantitative determination of glutathione by the use of iodine titration and reported the amounts present in a number of tissues.

The constitution of glutathione as a dipeptide of glutamic acid and cysteine capable of existing in either the —SH or —S—S— form was confirmed by Quastel, Stewart and Tunnicliffe[20], and by Johnson and Voegtlin[21]. These latter workers also showed[22] that injection of glutathione into animals inhibited the toxic action of arsenic and[23] exerted a protective action against cyanide. They also believed[24] that the toxic action of copper and gold might be a special type of asphyxia due to disturbance of the glutathione oxidation-reduction mechanism.

Hunter and Eagles[25] in 1927 modified Hopkins' technique for the preparation of glutathione from yeast, liver and blood. Upon analysis the product they obtained yielded a higher percentage of total nitrogen and of amino nitrogen and a lower percentage of sulfur than did the compound of Hopkins'. After the publication of their paper Hopkins began a reinvestigation of the matter and in 1929[26] reported glutathione to be a tripeptide consisting of glutamic acid, cysteine and glycine. However, he did not establish the nature of the linkages of the amino acids in the compound. In his original method of isolation the glycine was split from glutathione by prolonged boiling in water.

At about the same time of Hopkins' work Kendall and associates[27] began the publication of a series of papers on the isolation and identification of glutathione. In the first paper they reported that the material prepared by a modification of Hopkins' original method was a tripeptide of glutamic acid, glycine and cysteine with glycine attached to one carboxyl of glutamic acid and cysteine to the other. In a later communication they showed glutathione to be either glutamyl-cysteinyl-glycine or glutamyl-glycyl-cysteine, and finally in the last paper they identified it as glutamyl-cysteinyl-glycine represented by the following formula:



γ Glutamyl-cysteinyl-glycine

Glutathione

The synthesis of glutathione was first accomplished by Harington and Mead(28) in 1935.

Properties of Glutathione

Physical Properties. Glutathione is a white, crystalline compound melting at 190° C. It is non-hydroscopic when pure and is soluble to the extent of about 1 part to 10 parts of water at 0° C. It is easily soluble in warm water. The optical rotation of a 2 per cent solution of glutathione is $[\alpha]_D^{27} = -21.3°$.

Chemical Properties. Reduced glutathione is precipitated by the salts of heavy metals such as mercury, copper, lead, uranium, gold, silver and cadmium. Precipitation of glutathione with cadmium has been used in its quantitative determination to separate it from impurities in blood and tissue filtrates.

Reduced glutathione gives a cherry red color with sodium nitroprusside in the presence of ammonium hydroxide.

The sulfhydryl radical, —SH, gives the molecule its reducing properties. The hydrogen atom of —SH can be transferred to molecular oxygen in the presence of alkali and traces of iron and copper, or of iron porphyrin compounds.

In the oxidation of glutathione the H atoms are removed from two molecules and the glutathione residues are united by —S—S— constituting oxidized glutathione. If we represent the reduced form of glutathione by G SH then the oxidation proceeds as follows:

$$\text{G SH} + \underset{\text{O}}{\text{HS}}\text{—G} \longrightarrow \text{G—S—S—G}$$

The oxidation of glutathione may be accomplished readily with a variety of oxidizing agents among which iodine is most commonly used for analytical purposes:

$$\text{G SH} + \underset{I_2}{\text{HS}}\text{—G} \longrightarrow \text{G—S—S—G} + 2\text{HI}$$

The oxidised form of glutathione

Arsenophosphotungstic acid and potassium ferricyanide also oxidize glutathione.

The oxidized form of glutathione is reduced by hydrogen cyanide, zinc dust, and hydrogen sulfide. These compounds have been used in determining the amount of oxidized glutathione present in biological materials.

Determination of Glutathione

The oxidation of glutathione as described in the preceding section has been used in a number of modifications by various workers.

Iodine titration of glutathione was the basis of the method for the quantitative determination reported by Tunnicliffe[18] in 1925 and by Murray[19] in 1926. Their values were calculated according to the composition of glutathione as cysteinyl-glycine reported by Hopkins.

The iodine oxidation method for the determination of glutathione in blood described by Woodward and Fry[29] has been widely used. These workers prepared a sulfosalicylic acid filtrate of blood containing 2 per cent of the acid as a final concentration and having a pH of about 2. Samples of filtrate were further acidified by the addition of more sulfosalicylic acid, starch indicator and potassium iodide were added, and the mixture titrated to a blue color with standard potassium iodate. It was found that glutathione in filtrates at pH 2 and lower did not undergo autooxidation.

Mason[30] pointed out that substances in blood and tissues other than glutathione give high values by iodine oxidation methods, and that such methods yield variable results unless the conditions are very carefully controlled. He described a method for determining glutathione based upon oxidation with ferricyanide at pH 5.9, followed by conversion of the ferrocyanide formed to Prussian blue and estimation of the Prussian blue colorimetrically.

Gabbe[31] at about the same time used ferricyanide in acid solution for oxidation of glutathione. He suggested the use of an index for glutathione in relation to the number of red blood cells, $\frac{\text{Glutathione}}{\text{RBC}}$, which, in some cases, has been adopted by other workers.

Benedict and Gottschall(32) developed a method for the determination of glutathione utilizing arsenophosphotungstic acid as the oxidizing agent on protein-free blood filtrates made with tungstomolybdic acid. The colored reduction product of arsenophosphotungstic acid formed in the oxidation was estimated colorimetrically. They reported that determinations on blood using Mason's method showed glutathione values averaging about 6 mgs. per cent higher than with their method.

Binet and Weller(33, 34) prepared trichloracetic acid filtrates of blood and tissues, precipitated the glutathione as the cadmium salt with cadmium lactate, which was then washed and dissolved in acid. The acid solution was treated with iodide and standard iodate, and the excess iodine above that required for oxidation of glutathione was titrated with thiosulfate.

In 1947 Brückmann and Wertheimer(35) reported a method for the determination of glutathione based upon the color reaction between sulfhydryl compounds and sodium nitroprusside in the presence of alkali and ammonium ions. The color develops and fades rapidly but by the use of a spectrophotometer it is possible to obtain reliable results.

Values for the glutathione contents of blood and tissues vary somewhat with different methods of determination due to interfering substances. Table I shows some of the values for glutathione in blood and tissues reported by different workers.

Variations of Glutathione with Age

In 1940 McNamara and Senn[38] published the results of a study of the glutathione content of blood in infancy and childhood. They found that at birth the blood glutathione is higher than in adults, but falls rapidly during the first two to three weeks and slowly until about the third month when it is below adult levels. The blood glutathione levels of children remain lower than those of adults through the eleventh year. The red blood cell count follows the same course as glutathione until about the second month after which it rises gradually to normal adult values.

Binet and Poutonnet[39] studied the glutathione content in the blood and tissues of young rats four months old, and old rats more than two years old. They reported the glutathione values for blood, muscle and kidney of the old rats to be higher than the values for the young rats, while the values for liver were not significantly different. They also reported the glutathione content of the blood of old people to be relatively high.

Relation of Glutathione to Anemia

Gabbe[31] reported a lowering of the glutathione content of

Table I

Reduced Glutathione in Blood and Tissues

Workers	Species	Blood†	Liver*	Muscle*	Kidney*	Heart*	Brain*	Adrenals*
Woodward and Fry(28)	human	34						
Mason(30)	hog	22						
	rabbit	29		42	55			
Gabbe(31)	human	35						
Benedict and Gottschall(32)	human	36						
Binet and Weller(36)	rat	27	177	33	145	73	74	
	guinea pig	38	229	32	118	73	67	130
	rabbit	29	268	44	111	73		121
	dog	24	171	37		77		112
Brückmann and Wertheimer(35)	rat	37	176		109			
Goffart and Fischer(37)	rabbit	31	267	69	188			

† Mg./100 ml.

* Mg./100 gm.

the blood in pernicious anemia parralleling the drop in red blood cells. However, the concentration of glutathione in the cells of pernicious anemia patients was higher than in cells of normal persons. During remissions in pernicious anemia the blood glutathione values rose with the red blood cells and the glutathione content of the cells fell to the normal range.

Litarczek, Baicoiano and Bals[40] reported that in rabbits made anemic by bleeding the glutathione content of the corpuscles is increased.

Morrison and Williams[41] found that glutathione or cysteine is efficient, within physiological pH range, in reducing methemoglobin to hemoglobin, and suggested that glutathione is a part of the mechanism which prevents the accumulation of methemoglobin in the intact red blood cell.

Variations of Glutathione with Diet

Blanchetière and Binet[42] in 1926 studied the effect of diet on the glutathione content of the tissues of dogs. They fed two diets, one of which consisted of water, carbohydrate and fat, the other of water, carbohydrate, and meat free of fat. They found no variation in the glutathione contents of the tissues except that the kidney and liver values were slightly lower on the fat diet.

Leaf and Neuberger[43] fed rats a low protein diet containing 83 per cent starch and 12 per cent fat. They reported that in fourteen days the liver glutathione content had dropped to 20 to 30 per cent of normal.

Griffiths[44] reported lowering the blood glutathione of rabbits from an initial value of 38 to 18 to 23 mg. per cent in six to seven weeks by feeding a diet low in cystine and methionine. The protein, arachin, low in these amino acids, was used by Griffiths. He also hastened the lowering of blood glutathione by eliminating protein from the diet.

Collins-Williams and Bailey[45] used the same methionine and cystine deficient diet as that employed by Griffiths and found it difficult to lower the blood glutathione of rabbits because the animals showed anorexia, loss of weight, marked loss of hair, anemia and general weakness. Nine of twenty-one rabbits died before the blood glutathione was lowered to 25 mg. per cent. Of the remaining 12 rabbits only 5 showed blood glutathione values of 22 mg. in 4 to 8 weeks, and 1 reached this level only after 12 weeks.

Schiff and Fukuyama[46] found the liver glutathione of young mice to be greatly reduced by dehydration resulting from a diet of dry milk and a minimum of water.

Hirano[47] found the reduced glutathione content of the blood to be decreased from 30 mg. per cent to 22 mg. per cent in starved rabbits.

Binet and Weller[48] studied the influence of inanition on the glutathione content of the tissues of guinea pigs. After eight days of starvation the liver glutathione level had dropped from 227 to 181 mg. per cent and the blood glutathione from 41 to 37 mg. per cent.

Prunty and Vass[49] reported that the concentration of glutathione in the blood of human beings varies inversely with that of the plasma ascorbic acid.

Grunert and Phillips[50] made use of a diet low in sodium to reduce the blood glutathione of rats. The rats were kept on a diet containing only 0.005 per cent sodium and 0.5 per cent potassium, and after ten to twelve weeks the glutathione values in the blood had dropped to an average of 8 mg. per cent.

Relation of Glutathione to Enzymes

Many enzymes of the body have been shown to contain sulfhydryl groups which are essential for their activity. Sumner and Poland[51] in 1933 found that crystalline urease gave a positive nitroprusside test and believed that the urease molecule itself contains sulfhydryl groups. Hellerman and associates[52] showed these sulfhydryl groups to be essential for the activity of urease.

Jowett and Quastel[53] studied the function of glutathione in the glyoxalase activity of the red blood cell. They postulate that lysed erythrocytes lose their glyoxalase activity due to dilution of glutathione, since they found a return of activity when glutathione was added to bring the content up to the original concentration present in the red cells. They suggested a reversible equilibrium between GSH and glyoxalase.

Sulfhydryl groups were reported present only in certain hydrolytic enzymes until their presence in oxidation enzymes was demonstrated in 1938 by Hopkins and Morgan[54] in succinoxidase, and by Rapkine[55] in phosphoglyceraldehyde oxidase. Hopkins and associates[56] showed that succinic dehydrogenase is inactivated by G—S—S—G and restored by G—SH, and also that this enzyme is inactivated by alloxan, copper, maleic acid and iodoacetic acid which react with —SH groups.

These findings and many others indicate a wide distribution of enzymes requiring the presence of —SH groups for activity.

Barron and Singer[57, 58] carried out an extensive study of the enzymes concerned with the metabolism of carbohydrates, fats and proteins and found many of these enzymes to contain essential —SH groups.

Relation of Glutathione to Diabetes

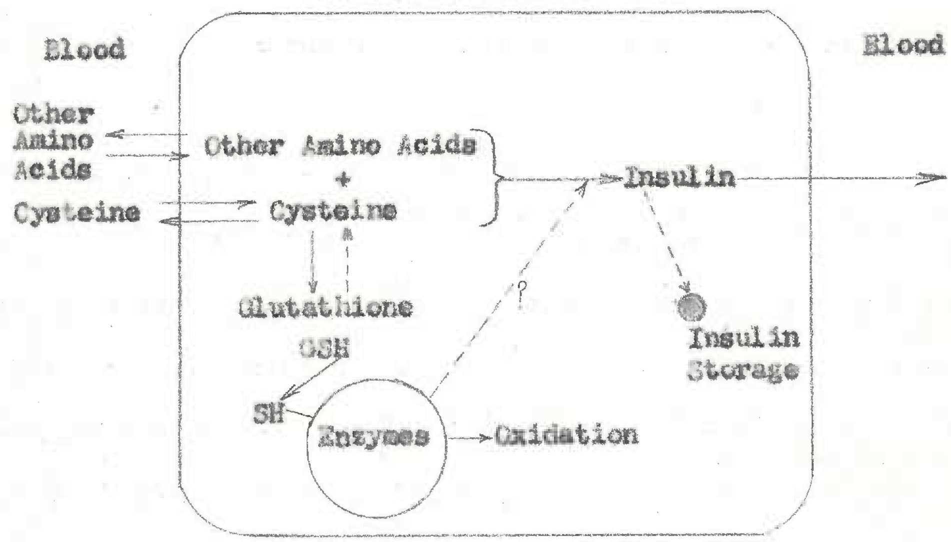
The relation of glutathione to the metabolism of the beta cells of the pancreas is of special interest. The formation of insulin by these cells requires relatively large amounts of sulfur containing amino acids in competition with the formation of glutathione. Also, glutathione appears to play an important role in the function of the beta cells.

Du Vigneaud[59] found that insulin contains a much higher percentage of cystine than most other proteins of the body which necessitates the availability of relatively large amounts of cysteine for insulin synthesis. As has been mentioned previously the activity of a great number of enzymes required in body metabolism depends upon free sulfhydryl groups, and activity is lost when the —SH groups are oxidized to —S—S—. By contrast the activity of insulin depends upon the sulfur being in the —S—S— form. According to du Vigneaud[60] addition of cysteine to insulin results in the reduction of —S—S— to —SH and completely inactivates it. Since the beta cells synthesize insulin in the —S—S— form they may have an oxidation-reduction potential adjusted to favor the oxidation of —SH to —S—S—. Bensley[61] selectively stained the islands of Langerhans of the pancreas with Janus green. The dye is reduced to its colorless form more rapidly by the ascinar cells than by the beta cells indicating a higher oxidation-reduction potential in the beta cells. The beta cells, accordingly, may have less reduced glutathione and other sulfhydryl compounds present in them than do other cells, and may have less ability to reduce certain toxic compounds such as alloxan to less toxic forms.

Lazarow[62] has postulated that as a result of the use of cysteine for insulin synthesis the amount of glutathione present in the beta cells may be decreased rendering the enzyme systems of the beta cells more susceptible to toxic substances. Any situation such as increased carbohydrate intake, administration of large amounts of anterior pituitary hormone, thyroid hormone, or glucose which leads to an increased demand for insulin, and increased stimulus for the synthesis of insulin, may bring about depletion of glutathione in the beta cells, cause them to be less resistant to toxic substances and lead to this degeneration.

Lazarow[62] presents the following diagram to summarize the metabolism of the beta cells.

Metabolism of Beta Cell

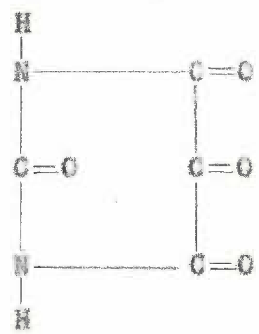


As has been stated previously insulin is inactivated by sulfhydryl groups. Lehmann[63] reported that insulin is inactivated in vitro by extracts of rabbit muscle and that two substances are responsible, a dialyzable, thermostable fraction, probably glutathione, and a non-dialyzable, thermolabile fraction, probably consisting of proteins containing —SH groups.

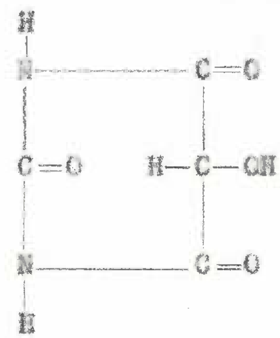
Levine, Hechter, Grossman and Soskin[64] found that the glutathione content of the livers of animals known to be hypersensitive to insulin is significantly lower than the glutathione content of normal animals.

The production of diabetes in animals by alloxan has been demonstrated by many investigators(62).

It has been shown by Archibald(65), Lazarow and Patterson(66), and Patterson, Lazarow and Levey(67) that alloxan administered to animals is partly destroyed by the glutathione of blood. Alloxan is reduced to dialuric acid by glutathione.



Alloxan



Dialuric acid

According to the concept of these workers some of the alloxan which escapes destruction in the blood passes to the beta cells of the pancreas and oxidizes —SH groups of enzymes and tissue proteins thereby causing loss of function, degeneration, and diabetes.

Leech and Bailey(68), working with rabbits, and Brückmann and Wertheimer(35) with rats, demonstrated that the blood glutathione content falls markedly immediately after administration of alloxan. Lazarow(69) found that glutathione or cysteine will protect against the action of alloxan if injected within a few minutes prior to administration of alloxan.

Goldner(70) and West and Highet(71) have reported guinea pigs to have high resistance to alloxan. Griffiths[72] found that the blood glutathione of guinea pigs is significantly higher than that of rabbits, but when guinea pigs were placed on a methionine-cystine deficient diet they showed diabetic tendencies after alloxan injection.

Griffiths[44] has reported the production of diabetes in rabbits by the injection of uric acid. The rabbits were first placed on a methionine-cystine deficient diet for 6 to 7 weeks during which time the blood glutathione fell from an average value of 38 to 18 to 23 mg. per cent. Collins-Williams and Bailey[45], using Griffiths' technique, showed a transitory hyperglycemia in only two of twelve rabbits. Griffiths considers that some intermediate metabolic product of uric acid, possibly resembling alloxan, may act upon the islet cells to cause diabetes when insufficient glutathione is present to detoxify it.

The injection of anterior pituitary preparations has been found by a number of workers[73, 74, 75, 76] to lower the glutathione content of blood and tissues. Recently Conn and associates[77, 78] have injected purified adrenocorticotropic hormone and have produced a transitory diabetes in man, which could be directly correlated with a fall in blood glutathione levels.

A number of reports relating to blood glutathione levels in human diabetes have appeared but little attention has been paid to age of patients, duration of diabetes, or treatment so that no finite conclusions can be drawn.

Glutathione Protection Against X-Radiation

In 1933 Woodward[79] irradiated pure solutions of glutathione with X-rays and found no effect, but Kinsey[80] in 1935 reported that X-rays have a destructive effect on glutathione solutions and that there is a linear relationship between the destruction of glutathione and the dose of X-radiation.

Ephrati[81] in 1948 found staphlococcus hemolysin to be destroyed by X-irradiation. He tried protective agents and found that glutathione was among the effective compounds.

Recently Patt and associates[82, 83, 84] have published a series of papers on protection against X-irradiation. They found cysteine and glutathione effective if administered before the animals were irradiated. Cysteine was effective if given either orally or intravenously. Glutathione was not effective orally, but was about one third as effective for a given weight as cysteine when given intravenously. Cystine and methionine gave no protection.

Cronkite and Chapman[85] exposed mice to various doses of X-irradiation. They found that adrenalectomy 10 days previously increased the sensitivity of mice to irradiation, and suggested that since the adrenalectomized animals showed low hepatic and total body reduced glutathione levels 10 days after adrenalectomy, the increased sensitivity may be related to sulfhydryl levels of tissues. They also injected a group of mice subcutaneously with glutathione (4 mgs. per gm.) immediately before irradiation and found the sensitivity to irradiation to be reduced.

Purpose of the Investigation

The research embodied in the first and second parts of this thesis was begun after the report of Griffiths[44] on the production of diabetes by injection of uric acid into rabbits with low blood glutathione levels. It was felt that an attempt should be made to repeat Griffiths' work using rats as experimental animals. While we have been unable to produce diabetes by the intraperitoneal injection of uric acid into rats with low blood glutathione values, we feel that we have a body of information relating to the variation of blood glutathione in rats with age and with different diets which is of some interest.

The third part of the research was undertaken after the report of Patt and associates[82]. These workers found rats to be afforded some protection from X-irradiation by cysteine previously administered. It seems reasonable to assume that this protective action of cysteine may be related to its capacity to keep essential enzyme systems in their —SH forms through its reducing properties. Since glutathione has reducing powers similar to those of cysteine and presumably functions in tissues to maintain enzymes in their active —SH forms, we considered that a study of the variation of liver and blood glutathione levels of rats after irradiation might reveal facts of interest.

EXPERIMENTAL WORK

Determination of Glutathione in Blood and Liver

The method of Brückmann and Wertheimer[35] utilizing the very sensitive reaction of glutathione with sodium nitroprusside is well suited for use with small amounts of material such as are obtainable from rats. The following modification was adapted for our use.

Apparatus:

Model 6 A Coleman Junior Spectrophotometer

Coleman selected cuvettes 19 x 105 mm.

Reagents:

Metaphosphoric acid. A 6 per cent aqueous metaphosphoric acid solution freshly prepared each day was used for precipitation of blood and tissue proteins. For known solutions and reagent blanks a 2 per cent aqueous solution of metaphosphoric acid saturated with sodium chloride was used.

Sodium chloride. Baker's analyzed C.P. grade, and a saturated solution prepared from it.

Sodium nitroprusside. A solution of 0.1 gm. of sodium nitroprusside in 10 ml. of 0.4 per cent $(NH_4)_2SO_4$. This reagent must be freshly prepared and used within 10 minutes.

Sodium carbonate. A solution of 25 per cent anhydrous Na_2CO_3 in water.

Known solutions of glutathione. A stock solution of 40 mg.

per cent glutathione in 2 per cent metaphosphoric acid saturated with NaCl was prepared, and from this dilutions of 1 to 8 mg. per cent in 2 per cent metaphosphoric acid saturated with NaCl were made. All glutathione solutions were used within 2 hours.

Procedure:

A curve was established for glutathione values by determination of spectrophotometer readings on solutions containing 1, 2, 3, 4, 6 and 8 mg. per cent glutathione. Duplicate determinations were set up as follows. 2 ml. of known solution and 1 ml. of saturated NaCl solution were placed in cuvettes. To each cuvette 0.5 ml. of sodium nitroprusside solution and 0.7 ml. of Na_2CO_3 solution were added. The solutions were mixed and the optical density read within 30 seconds in the spectrophotometer at 515 mμ. The spectrophotometer was set at 0 with a blank sample containing 2 ml. of 2 per cent metaphosphoric acid, 1 ml. of saturated NaCl solution, 0.5 ml. of sodium nitroprusside solution, and 0.7 ml. of sodium carbonate solution. A curve was plotted as illustrated in Chart I. The color develops rapidly and fades rapidly but with care reproducible results are obtained.

For the determination of reduced glutathione in blood 0.2 ml. of freshly drawn oxalated blood was laked in 1.5 ml. of water. To this solution 1 ml. of 6 per cent metaphosphoric acid and 1 gm. of sodium chloride were added. The mixture was shaken, allowed to stand 10 minutes, and then centrifuged for 15 minutes at 3,000 R.P.M. A small cotton filter was attached to the end of a 2 ml.

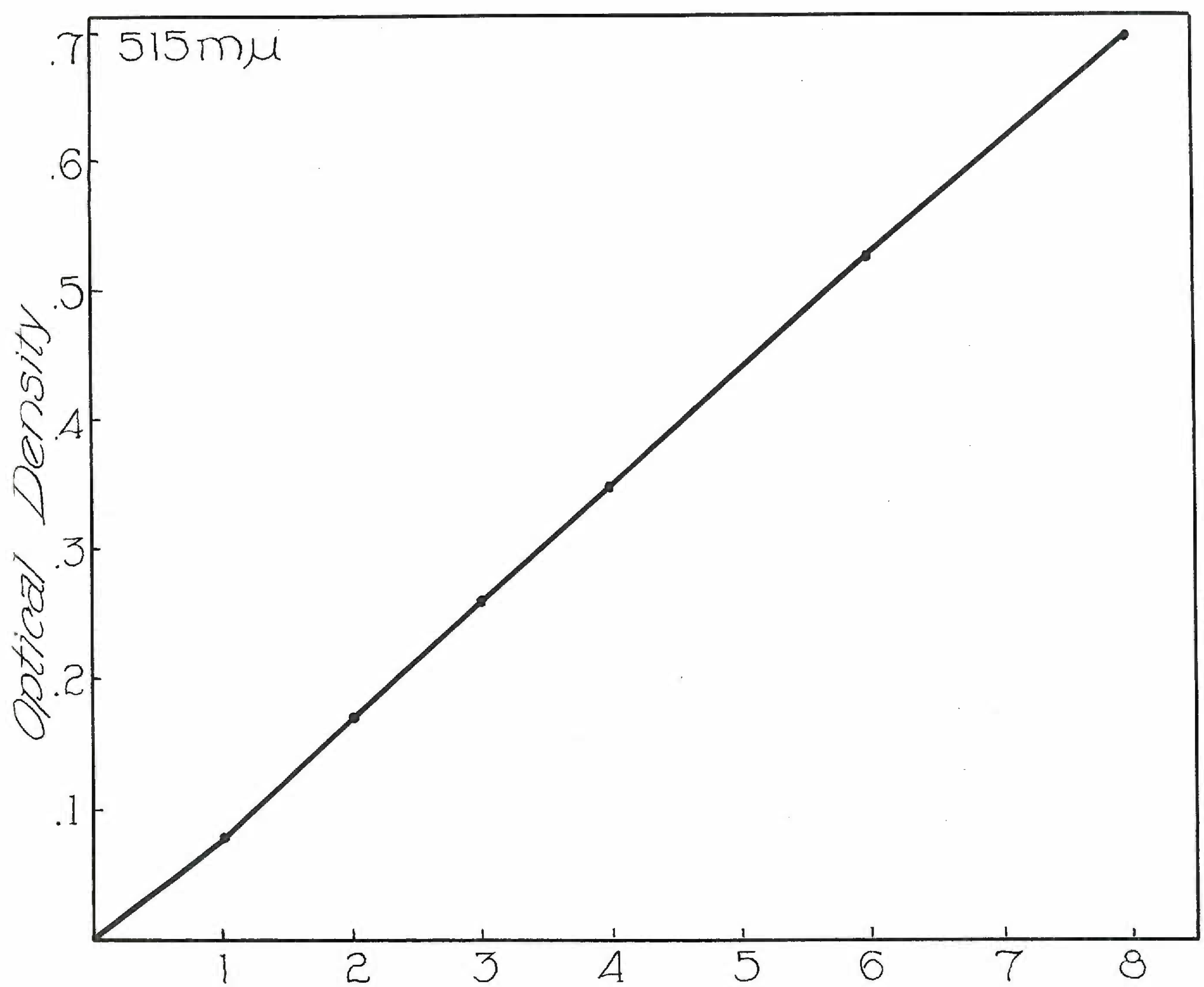


Chart I. Standard Solutions of Glutathione pipette by means of a small rubber band, 2 ml. of filtrate were transferred to a cuvette and treated in the same manner as the known solutions above. Values were read from the curve for known solutions and the glutathione contents calculated.

The determination of liver glutathione was carried out as follows. A rat was anesthetized with 3 mg. of nembutal per 100 gms. of rat, injected intraperitoneally. A solution of nembutal containing 6 mg. per ml. was used. It was sometimes necessary to give a small additional dose in order to obtain satisfactory anesthesia. A midline incision was made and a piece of liver weighing from 40-160 mg. was cut from the left lateral lobe, divided into two pieces, and placed in weighed tubes containing 1.5 ml. of water. The tubes were quickly reweighed, a little finely ground soft glass added, and the tissue macerated with a glass rod. The rod was rinsed with 1 ml. of 6 per cent metaphosphoric acid. To the mixture in each tube 1 gm. of sodium chloride was added, the tube stoppered and allowed to stand 10 minutes with occasional shaking after which it was centrifuged for 15 minutes at 2,600 R.P.M. 2 ml. of the supernatant solution were pipetted into a cuvette and glutathione estimated as outlined above.

Variation of the Blood Glutathione of Rats with Age

Blood glutathione levels were determined on rats of different ages from 7 to over 190 days. The 7 day old rats were decapitated for blood sampling. The same procedure was used for 3 of the 14 day old rats, while in the case of 3 others blood was taken from the vena cava. All other blood samples were obtained from the tails.

The data are summarized in Table II.

Table II

Blood Glutathione Values of Rats in Relation to Age

Ages after 28 days are approximate and estimated from weights.

Age Days	Blood Glutathione Mg. per 100 ml.	Average Mg. per 100 ml.
7	11, 12, 15, 12, 11, 7	11
14	6, 9, 18, 18, 13, 15	13
21	24, 23, 20, 21, 29	23
28	31, 29, 28, 22, 29, 25	27
35	33, 28, 33, 27	30
54	36, 42, 45, 31	38
61	39, 38, 38, 37, 40	38
80-190	Average for 114 rats	44
Over 190	56, 48, 53, 47, 50, 47, 47, 41	49

The above data indicate that the blood glutathione values are low in 7 day old rats and gradually increase to the average value of 44 mg. per cent for adult rats. In rats over six months old the values are slightly higher.

By the trained feeding of a mother rat it was possible to raise six rats to 21 days of age with no food other than their mother's milk. These rats showed lower blood glutathione values than those which had access to the regular diet of Purina chow. Values for the 6 rats raised on mother's milk to 21 days of age were 17, 15, 14, 18, 9, 9 mg. per cent, with an average value of 14 mg. per cent. The average value for 5 rats subsisting on mother's milk plus chow to 21 days of age was 23 mg. per cent. It is uncertain whether these variations are related to quantity or quality of the diet or to both.

Variation of Glutathione Levels in Rats on Different Diets

The following experiments were undertaken after the publication of Griffiths(44) on the production of diabetes by the injection of uric acid. He claimed to have produced diabetes in rabbits by the injection of uric acid after the blood glutathione levels of the animals had been lowered markedly by subsistence on a sulfhydryl deficient diet. We were unsuccessful in an attempt to render rats diabetic by the technique used by Griffiths on rabbits. However, results were obtained which are of interest in relation to the effect of diet upon blood glutathione levels.

Effects of Low Protein Diets. Six rats (Sprague-Dawley strain) were placed on a low protein diet of the following composition:

Casein	6.0	per cent
Yeast	5.0	" "
Salt mixture	4.0	" "
Cod liver oil	2.0	" "
Dextrin	66.8	" "
Glucose	8.0	" "
Wesson oil	8.0	" "
Vitamin C	0.2	" "

After one week on this diet blood glutathione levels of all six rats were above average values and it was considered advisable to change the diet to one containing peanut meal. The latter is rich in the protein, arachin, which is deficient in the sulfur containing amino acids. The composition of this diet was as follows:

Peanut meal	10.0	per cent
Dextrin	65.3	" "
Lard	19.0	" "
Cod liver oil	1.0	" "
Vitamin B complex mixture . . .	0.5	" "
Choline	0.1	" "
Salt mixture	4.0	" "
Vitamin C	0.1	" "

Blood glutathione levels were determined at about three week intervals. The data are summarized in Table III.

The blood glutathione levels of the rats rose during the 6 days on the 6 per cent casein diet, and did not drop to near normal until about the sixty-sixth day after which they remained normal until the ninety-third day when the experiment was terminated.

Additional information concerning the composition of his sulfhydryl deficient diet was made available to us by Griffiths(86), and a group of four rats was placed on the following diet:

Dextrin	76.3	per cent
Peanut meal	10.0	" "
Salt mixture	3.5	" "
Peanut oil.	5.0	" "

Table III

Effects of Low Protein Diet on Blood Glutathione Levels in Rats

Values for glutathione and weights represent milligrams per cent and grams, respectively.

Rat	Determination	Normal Diet	Days on Experimental Diet				
			6*	31	52	66	93
1	Glutathione		65	71	51	39	37
	Weight			280	256	246	257
2	Glutathione		62	62	62	62	41
	Weight			255	225	220	243
3	Glutathione		63	59	48	45	39
	Weight			210	194	190	205
4	Glutathione		51	65	54	45	39
	Weight			215	200	190	212
5	Glutathione		61	70	64	57	52
	Weight			260	246	240	260
6	Glutathione		57	51	54	41	43
	Weight			225	200	196	201
	Glutathione Average	44†	60	63	56	48	42

*Values after 6 days on 6 per cent casein diet.

†Average of 114 rats.

Cod liver oil	4.0 per cent
Wheat germ oil	0.7 " "
Ascorbic acid	0.1 " "
Vitamin B complex mixture . . .	0.2 " "
Choline	0.2 " "

Table IV summarizes the results obtained in rats maintained on this diet for 119 days.

It may be seen from these data that the first drop in blood glutathione values was found after the rats had been on the above diet for almost 2 months. After 119 days the one surviving rat had a blood glutathione value of 18 mg. per cent. Rats 3 and 4 showed an anomalous rise in blood glutathione at 78 days.

Effects of a Protein-Free Diet. Griffiths[44] reported that in the case of one rabbit lowering of the blood glutathione level was accomplished in less time by entirely eliminating protein from the diet for 2 weeks. In view of this report we placed 8 rats on a protein-free diet as follows:

Lard	16.0 per cent
Cod liver oil	1.0 " "
Salt mixture	4.0 " "
Dextrin	74.75 " "
Glucose	4.0 " "
Vitamin B complex	0.05 " "
Choline	0.1 " "
Vitamin C	0.1 " "

Table IV

Effects of Sulfhydryl Deficient Diet on Blood Glutathione Levels in Rats

Values for glutathione, hematocrit and weight represent milligrams per cent, per cent, and grams, respectively.

Rat	Determination	Initial Values	Days on Experimental Diet							
			9	24	38	53	65	78	84	119
	Glutathione	46	38	37	44	32	25	31	24	18
1	Hematocrit	43	47	47	41	38	40	36	35	25
	Weight	185	136	140	135	128	126	122	114	102
	Glutathione	41	39	45	41	35	31	34		
2	Hematocrit	46	47	—	—	44	42	34	dead	
	Weight	205	178	196	193	194	170	158		
	Glutathione	49	42	52	41	39	29	41	33	
3	Hematocrit	—	48	42	37	40	44	42	35	dead
	Weight	227	177	196	193	196	194	192	186	
	Glutathione	41	37	44	41	37	27	49		
4	Hematocrit	—	42	43	40	43	43	44	dead	
	Weight	212	190	207	200	180	195	175		

After the animals had been maintained on this diet for 37 days they had lost weight (in some cases more than half of their original body weight) and hair and were in generally poor condition. Four rats died after about one month. The blood glutathione levels were not significantly lowered. A resume of the data for this series will be found in Table V.

Effects of an Ascorbic Acid Diet. The findings of Prunty and Vass[49] that the concentration of glutathione in the blood of human beings varies inversely with that of the plasma ascorbic acid led us to try the effect of a diet high in ascorbic acid. The diet consisted of 5 per cent ascorbic acid and 95 per cent Purine chow. After 18 days on this diet the blood glutathione values were unchanged (Table VI).

Effects of a Resin Diet. Grunert and Phillips[50] have reported that a diet low in sodium (below 0.005 per cent) is effective in lowering glutathione in the blood of rats. Because of the difficulty of obtaining diet constituents sufficiently low in sodium we found it difficult to prepare a satisfactory diet. Since Dock[87] has reported that cation-exchange resins are of value in withdrawing sodium from the body we decided to try the effects of these resins in relation to blood glutathione levels. Two Rohm and Haas Co. amberlite cation-exchange resins were selected for use, IR 50 and IR 100, and two rats were maintained on a diet prepared from each resin. The diets consisted of 10-20 per cent resin, 50 per cent Lonalac (Mead, Johnson and Co.) as a source of protein, and the

Table V

Effects of Protein-Free Diet on Blood Glutathione

Levels in Rats

Values for glutathione and weights represent milligrams per cent and grams, respectively.

Rat	Determination	Initial Values	Days on Experimental Diet		
			7	21	45
1	Glutathione	46	51	46	33
	Weight	168	146	118	107
2	Glutathione	49	50	49	39
	Weight	160	125	106	98
3	Glutathione	44	58	48	—
	Weight	146	120	98	95
4	Glutathione	48	44	45	39
	Weight	154	132	108	96
5	Glutathione	33	41	38	dead
	Weight	103	79	60	
6	Glutathione	28	32	34	—
	Weight	100	78	61	53
7	Glutathione	33	33	57	—
	Weight	103	76	56	52
8	Glutathione	27	34	23	30
	Weight	117	88	66	53

Table VI

Effects of a Diet Containing 5 Per Cent Ascorbic Acid on Blood Glutathione Levels in Rats

Values for glutathione and weights represent milligrams per cent and grams, respectively.

Rat	Determination	Initial Values	Days on Experimental Diet		
			13	20	31
1	Glutathione	49	42	45	50
	Weight	200			204
2	Glutathione	47	42	46	49
	Weight	186			190
3	Glutathione	41	31	45	37
	Weight	218			276
4	Glutathione		47	50	42
	Weight				330

remainder powdered sugar (0.02 per cent sodium). Lonalac contains 38 per cent carbohydrate, 28 per cent fat, 27 per cent protein, and 0.02 per cent sodium. It is a milk product treated to remove most of the sodium.

Since the diets were relatively low in sodium it was anticipated that the action of the resin would induce negative sodium balances in the rats.

The data of Table VII show that on these resin diets the blood glutathione levels fell gradually in all of the rats until after about 7 weeks all but 1 rat showed a value below 30 mg. per cent. The animals gradually lost weight and were in generally poor condition. The experiment was terminated when one rat from each series died and the other rat ate part of his body.

It will be seen from the experiments in this section that it is very difficult to obtain low blood glutathione values in rats by variations in diet. In no case were we able to do so without severe loss of body weight and severe general deterioration in the health of the animals.

Table VII

Effect of Diets Containing Ion-Exchange Resins on Blood Glutathione Levels in Rats

Values for glutathione, hematocrit and weight represent milligrams per cent, per cent, and grams, respectively.

Resin	Rat	Determination	Initial Values	Days on Experimental Diet					
				8	12	24	31	43	
IR 50	1	Glutathione	39	25	41	39	49	28	
		Hematocrit	42	52	53	48	44	45	
		Weight	210	162	176	175	181	164	
	2	Glutathione	34	39	42	34	37	24	
		Hematocrit	42	51	52	50	46	44	
		Weight	250	182	177	170	157	140	
				Days on Experimental Diet					
				9	19	23	35	42	54
IR 100	1	Glutathione	37	46	32	40	27	30	24
		Hematocrit	44	49	48	46	32	33	41
		Weight	245	224	227	232	190	212	156
	2	Glutathione	42	45	38	31	37	38	35
		Hematocrit	44	41	49	33	50	38	36
		Weight	255		236	218	228	213	212

Variation of the Blood and Liver Glutathione of Rats After Irradiation

The following research was begun after the report of Patt and associates[82] showing the protective action against irradiation of previously administered cysteine. It was considered that facts of interest might be revealed by a study of blood glutathione levels after total body irradiation. When it became evident that marked changes in blood glutathione levels occurred it seemed advisable to determine glutathione in the livers of the rats since synthesis of glutathione takes place in the liver.

Male and female rats (Sprague-Dawley) weighing from 170 to 280 gms. were used in series of 10 to 12 animals.

Although the quantity of blood drawn for determinations amounted to only 2 per cent of the estimated volume, initial blood glutathione values were determined 1 week before irradiation to allow time for recovery of blood loss. Little more than 0.2 ml. of freshly drawn oxalated tail blood was used for glutathione and hematocrit determinations.

For irradiation, the rats of the first two series of 12 rats each were placed in a shallow metal pan 9 inches square, 6 at a time, and subjected to 500 r total body irradiation. The radiation factors were: 200 kv., 15 ma., 0.5 mm. Cu + 1 mm. Al filters, 50 cm. target distance, 1.28 mm. Cu half-value layer, 60.8 r per minute dose rate, 8 minutes and 14 seconds time of irradiation. Total body irradiation of 500 r is well below the LD_{50} dose of 640 ± 5 r reported by Clark and Uncapher[88].

The possibility that there might be lack of uniformity in the dose of irradiation due to scattering from the metal pan caused us to change to a plywood box for the remaining 4 series. A round box was constructed which held 10 rats in separate V-shaped compartments with the rats heads at the center of the box. Because of the size of the box it was necessary to lengthen the target distance for irradiation and to lengthen proportionately the time of exposure. The radiation factors then became: 200 kv., 15 ma., 0.5 mm. Cu + 1 mm. Al filters, 80 cm. target distance, 1.28 mm. Cu half-value layer, 24 r per minute dose rate, 20 minutes and 48 seconds time of irradiation.

Blood was drawn for analysis at various intervals of time after irradiation. In the first series determinations were begun ten minutes after irradiation. Because of the possible effect of blood loss it was considered that only one determination a day per rat would yield valid results. In subsequent series the time interval between irradiation and sampling was lengthened since no change was observed in early determinations.

In series II (rats 11-24) a few samples of liver were taken from rats with very low blood glutathione values and analyzed for glutathione. In series III (rats 25-34) all animals were biopsied for liver determinations after irradiation. Control liver biopsies were done on all of the animals of series IV, V, and VI (rats 35-58).

The animals were allowed a recovery period of 2 weeks after biopsy, then blood glutathione levels were run, and after 1 more week the rats were irradiated. In no case was it considered advisable to do more than 1 biopsy per rat following irradiation, nor were blood glutathione values considered significant after biopsy until they began to rise toward control levels.

After irradiation the rats developed diarrhea, appeared to have irritated respiratory tracts, gradually lost weight, and in some cases developed rough coats and were in poor general condition.

Since inanition has been reported[48] to cause low tissue glutathione values it was considered advisable to run liver glutathione determinations on a series of animals subjected to starvation. This series indicated that much greater weight loss than took place in the irradiated rats must occur before liver glutathione levels drop below the control range.

Liver glutathione values obtained at 2 week intervals on normal animals always fell well within the control range.

Glutathione determinations made on the plasma of a number of animals during the post-irradiation period showed complete absence of the substance.

Red blood cell glutathione concentrations were calculated by dividing the glutathione value for the whole blood by the hematocrit times 100.

The results of the work outlined above are presented in Table VIII.

Table VIII

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent			Hematocrit			Calculated Red Blood Cell Glutathione Mgs. per cent			Weight Gms.			Liver Glutathione Mgs. per cent
1	41 (i)	44 (10m)	30 (3d)	— (i)	40 (10m)	28 (3d)	— (i)	110 (10m)	107 (3d)	258 (i)	273 (10m)	216 (3d)	
2	39 (i)	44 (15m)	41 (3d)	46 (i)	43 (15m)	40 (3d)	85 (i)	102 (15m)	102 (3d)	216 (i)		187 (3d)	
3	41 (i)	50 (20m)	43 (3d)	48 (i)	41 (20m)	39 (3d)	85 (i)	122 (20m)	110 (3d)	229 (i)	227 (20m)	184 (3d)	
4	49 (i)	49 (25m)	42 (3d)	46 (i)	40 (25m)	37 (3d)	106 (i)	122 (25m)	114 (3d)	203 (i)	207 (25m)	177 (3d)	
5	46 (i)	47 (30m)	37 (3d)	43 (i)	42 (30m)	34 (3d)	107 (i)	112 (30m)	109 (3d)	225 (i)	228 (30m)	198 (3d)	
6	41 (i)	41 (35m)		42 (i)	43 (35m)		98 (i)	95 (35m)		217 (i)	241 (35m)		

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent	Hematocrit	Calculated Red Blood Cell Glutathione Mgs. per cent	Weight Gms.	Liver Glutathione Mgs. per cent
7	45 (i), 40 (3h), 28 (1d), 28 (3d), 8 (6d)	43 (i), 42 (3h), 29 (1d), 24 (3d), 13 (6d)	104 (i), 95 (3h), 97 (1d), 117 (3d), 62 (6d)	206 (i), 198 (3d), 180 (6d)	
8	47 (i), 36 (3h), 43 (1d)	40 (i), 43 (3h), 38 (1d)	117 (i), 84 (3h), 113 (1d)	242 (i)	
9	44 (i), 46 (1h), 38 (1d)	44 (i), 40 (1h), 33 (1d)	100 (i), 115 (1h), 115 (1d)	187 (i)	
10	47 (i), 45 (1h), 51 (1d), 31 (6d)	41 (i), 42 (1h), 39 (1d), 25 (6d)	115 (i), 107 (1h), 131 (1d), 124 (6d)	240 (i), 200 (6d)	
11	39 (i), 43 (3h), 41 (1d), 24 (6d)	38 (i), 38 (3h), 36 (1d), 28 (6d)	103 (i), 113 (3h), 114 (1d), 86 (6d)	215 (i), 160 (6d)	

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent	Hematocrit	Calculated Red Blood Cell Glutathione Mgs. per cent	Weight Gms.	Liver Glutathione Mgs. per cent
12	39 (i) 38 (1h) 42 (1d)	45 (i) 40 (1h) 38 (1d)	87 (i) 95 (1h) 110 (1d)	211 (i)	
13	54 (i) 49 (6h) 50 (3d) 54 (6d) 34 (8d)	47 (i) 42 (6h) 41 (3d) 61 (6d) 28 (8d)	115 (i) 117 (6h) 122 (3d) 89 (6d) 121 (8d)	235 (i) 240 (6h) 215 (3d) 217 (6d) 225 (8d)	
14	51 (i) 30 (6h) 31 (3d) 29 (6d) 21 (8d)	45 (i) 35 (6h) 37 (3d) 32 (6d) 19 (8d)	113 (i) 86 (6h) 84 (3d) 91 (6d) 110 (8d)	220 (i) 213 (6h) 194 (3d) 190 (6d) 196 (8d) 184 (9d)	91 (9d)
15	54 (i) 54 (2d) 47 (5d) 42 (8d)	47 (i) 48 (2d) 40 (5d) 32 (8d)	115 (i) 113 (2d) 118 (5d) 131 (8d)	210 (i) 227 (2d) 219 (5d)	
16	51 (i) 53 (2d) 37 (5d)	45 (i) 46 (2d) 38 (5d)	113 (i) 115 (2d) 97 (5d)	240 (i) 210 (2d) 194 (5d)	

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat.	Blood Glutathione Mgs. per cent	Hematocrit	Calculated Red Blood Cell Glutathione Mgs. per cent	Weight Gms.	Liver Glutathione Mgs. per cent
17	43 (i), 42 (1d), 45 (4d), 14 (7d)	43 (i), 41 (1d), 38 (4d), 13 (7d)	100 (i), 102 (1d), 118 (4d), 108 (7d)	224 (i), 220 (1d), 199 (4d), 176 (7d)	
18	53 (i), 47 (2d), 44 (5d), 38 (8d)	48 (i), 46 (2d), 39 (5d), 37 (8d)	110 (i), 102 (2d), 113 (5d), 103 (8d)	220 (i), 210 (2d), 199 (5d), 224 (8d)	
19	53 (i), 41 (6h), 44 (3d), 39 (6d)	44 (i), 19 (6h), 38 (3d), 69 (6d)	120 (i), 84 (6h), 116 (3d), 57 (6d)	172 (i), 193 (6h), 183 (3d), 169 (6d)	
	26 (8d), 21 (14d), 36 (21d), 38 (30d)	33 (8d), 19 (14d), 41 (21d), 32 (30d)	79 (8d), 110 (14d), 88 (21d), 116 (30d)	177 (8d), 185 (14d), 146 (21d), 175 (30d)	
	30 (43d), 42 (50d)	41 (43d), 43 (50d)	73 (43d), 98 (50d)	174 (32d), 203 (43d), 202 (50d)	203 (32d)
20	48 (i), 46 (1d), 50 (4d), 39 (7d)	47 (i), 42 (1d), 38 (4d), 39 (7d)	102 (i), 109 (1d), 132 (4d), 100 (7d)	216 (i), 238 (1d), 226 (4d), 245 (7d)	
	32 (9d), 5 (14d), 39 (21d), 38 (30d)	20 (9d), 6 (14d), 37 (21d), 47 (30d)	162 (9d), 83 (14d), 105 (21d), 81 (30d)	217 (9d), 195 (14d), 164 (21d), 208 (30d)	
	34 (43d), 41 (50d)	44 (43d), 42 (50d)	77 (43d), 98 (50d)	242 (43d), 250 (50d)	

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent				Hematocrit				Calculated Red Blood Cell Glutathione Mgs. per cent				Weight Gms.				Liver Glutathione Mgs. per cent
21	48 (i)	37 (1d)	43 (4d)	38 (7d)	47 (i)	42 (1d)	34 (4d)	32 (7d)	102 (i)	88 (1d)	126 (4d)	119 (7d)	199 (i)	210 (1d)	168 (4d)	205 (7d)	
	24 (9d)	17 (14d)	39 (21d)	38 (30d)	21 (9d)	16.5 (14d)	40 (21d)		114 (9d)	103 (14d)	98 (21d)		188 (9d)	196 (14d)	159 (21d)	188 (30d)	
	31 (43d)				42 (43d)				74 (43d)				175 (43d)				85 (43d)
22	55 (i)	51 (2d)	47 (5d)		43 (i)	45 (2d)	39 (5d)		128 (i)	113 (2d)	120 (5d)		188 (i)	190 (2d)	167 (5d)		
23	52 (i)	41 (1d)	50 (4d)	34 (7d)	47 (i)	41 (1d)	40 (4d)	30 (7d)	110 (i)	100 (1d)	125 (4d)	113 (7d)	222 (i)	219 (1d)	196 (4d)	209 (7d)	
	10 (9d)				7 (9d)				139 (9d)				168 (9d)				50 (9d)
24	53 (i)	52 (6h)	50 (3d)	40 (6d)	47 (i)	46 (6h)	41 (3d)	46 (6d)	113 (i)	113 (6h)	122 (3d)	87 (6d)	190 (i)	199 (6h)	172 (3d)		
	20 (8d)				20 (8d)				100 (8d)				166 (8d)				

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent	Hematocrit	Calculated Red Blood Cell Glutathione Mgs. per cent	Weight Gms.	Liver Glutathione Mgs. per cent
25	41 (i), 61 (2d)	45.5 (2d)	134 (2d)	202 (i), 185 (2d)	166 (2d)
26	45 (i), 7 (9d)	13 (9d)	54 (9d)	195 (i), 163 (9d)	89 (9d)
27	35 (i), 49 (2d)	36 (2d)	136 (2d)	207 (i), 197 (2d)	201 (2d)
28	45 (i), 60 (3d), 31 (10d)	44 (3d), 21 (10d)	136 (3d), 148 (10d)	213 (i), 193 (3d), 185 (10d)	211 (3d), 162 (10d)
29	40 (i), 61 (3d)	37 (i), 47 (3d)	108 (i), 130 (3d)	220 (i), 205 (3d)	189 (3d)
30	50 (i), 19 (9d)	40 (i), 8 (9d)	125 (i), 238 (9d)	208 (i), 186 (9d)	211 (9d)

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat.	Blood Glutathione Mgs. per cent	Hematocrit	Calculated Red Blood Cell Glutathione Mgs. per cent	Weight Gms.	Liver Glutathione Mgs. per cent
31	42 (i) 43 (5d)	45 (i) 40 (5d)	93 (i) 108 (5d)	193 (i) 167 (5d)	197 (5d)
32	53 (i) 44 (5d)	41 (i) 31 (5d)	129 (i) 142 (5d)	210 (i) 179 (5d) 183 (7d)	205 (5d)
33	50 (i) 29 (7d)	41 (i) 23 (7d)	122 (i) 126 (7d)	203 (i) 155 (7d)	151 (7d)
34	52 (i) 28 (10d)	37 (i) 21 (10d)	140 (i) 134 (10d)	218 (i) 193 (10d)	182 (10d)
35	42 (i) 33 (8d)	46.5 (i) 30 (8d)	90 (i) 110 (8d)	240 (i) 235 (8d)	199 (i) 204 (8d)
36	41 (i) 32 (8d)	45 (i) 30 (8d)	91 (i) 107 (8d)	234 (i) 223 (8d)	190 (i) 180 (8d)

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent			Hematocrit			Calculated Red Blood Cell Glutathione Mgs. per cent			Weight Gms.			Liver Glutathione Mgs. per cent	
37	49 (i)	43 (2d)	46 (19d)	44 (i)	39 (2d)	32 (19d)	111 (i)	110 (2d)	144 (19d)	240 (i)	218 (2d)	226 (19d)	204 (i)	180 (2d)
38	47 (i)	41 (6d)		46 (i)	38 (6d)		102 (i)	108 (6d)		229 (i)	203 (6d)		184 (i)	226 (6d)
39	47 (i)	34 (6d)	40 (19d)		40 (6d)	34 (19d)		85 (6d)	118 (19d)	232 (i)	205 (6d)	230 (19d)	205 (i)	199 (6d)
40	47 (i)	39 (6d)		36 (i)	39 (6d)		131 (i)	100 (6d)		275 (i)	216 (6d)		176 (i)	148 (6d)
41	47 (i)	42 (8d)		46 (i)	35 (8d)		102 (i)	120 (8d)		267 (i)	223 (8d)		182 (i)	169 (8d)
42	41 (i)	47 (2d)		47 (i)	44 (2d)		87 (i)	107 (2d)		279 (i)	246 (2d)		179 (i)	183 (2d)

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent			Hematocrit			Calculated Red Blood Cell Glutathione Mgs. per cent			Weight Gms.			Liver Glutathione Mgs. per cent	
43	47	69		41	39		115	177		254	229		201	189
	(i)	(2d)		(i)	(2d)		(i)	(2d)		(i)	(2d)		(i)	(2d)
44	36	16		38	16		95	100		279	229		166	192
	(i)	(14d)		(i)	(14d)		(i)	(14d)		(i)	(14d)		(i)	(14d)
45	45	25		41	17		110	147		250	216		201	222
	(i)	(9d)		(i)	(9d)		(i)	(9d)		(i)	(9d)		(i)	(9d)
46	37	20	4.2	33	14	3.8	112	142	110	257	258	207	201	219
	(i)	(9d)	(13d)	(i)	(9d)	(13d)	(i)	(9d)	(13d)	(i)	(9d)	(13d)	(i)	(9d)
47	40	43	18	40	45	18	100	96	100	242	233	211	182	206
	(i)	(2d)	(12d)	(i)	(2d)	(12d)	(i)	(2d)	(12d)	(i)	(2d)	(12d)	(i)	(12d)
48	38	43	32	36	45	32	106	96	100	257	257	274	191	191
	(i)	(2d)	(14d)	(i)	(2d)	(14d)	(i)	(2d)	(14d)	(i)	(2d)	(14d)	(i)	(14d)

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent			Hematocrit			Calculated Red Blood Cell Glutathione Mgs. per cent			Weight Gms.			Liver Glutathione Mgs. per cent	
49	45 (i)	18 (8d)		45 (i)	7 (8d)		100 (i)	257 (8d)		250 (i)	216 (8d)		162 (i)	188 (8d)
50	47 (i)	52 (2d)	14.5 (12d)	41 (i)	43 (2d)	8 (12d)	115 (i)	121 (2d)	181 (12d)	243 (i)	237 (2d)	179 (12d)	163 (i)	184 (12d)
51	52 (i)	48 (2d)	16 (19d)	46 (i)	44 (2d)	10 (19d)	113 (i)	109 (2d)	160 (19d)	258 (i)	254 (2d)	196 (19d)	181 (i)	204 (19d)
52	41 (i)	14 (8d)		42 (i)	9 (8d)		98 (i)	156 (8d)		202 (i)	167 (8d)		191 (i)	126 (8d)
53	43 (i)	44 (2d)	8 (9d)	43 (i)	44 (2d)	5 (9d)	100 (i)	100 (2d)	160 (9d)	182 (i)	198 (2d)	176 (9d)	189 (i)	106 (9d)
54	37 (i)	32 (8d)		42 (i)	31 (8d)		88 (i)	103 (8d)		222 (i)	176 (8d)		168 (i)	254 (8d)

Table VIII (Cont.)

Effects of 500 r X-Irradiation on Rats

The numbers in parentheses represent the time of drawing samples: (i) initial value one week before irradiation, and (m) minutes, (h) hours, and (d) days after irradiation.

Rat	Blood Glutathione Mgs. per cent		Hematocrit		Calculated Red Blood Cell Glutathione Mgs. per cent		Weight Gms.		Liver Glutathione Mgs. per cent	
55	45 (i)	43 (1d)	42 (i)	41 (1d)	107 (i)	105 (1d)	210 (i)	217 (1d)	194 (i)	
56	44 (i)	47 (1d)	43 (i)	40 (1d)	102 (i)	118 (1d)	211 (i)	208 (1d)	181 (i)	
57	52 (i)	10 (8d)	46 (i)	6 (8d)	113 (i)	167 (8d)	214 (i)	184 (8d)	164 (i)	116 (8d)
58	49 (i)	47 (2d)	44 (i)	40 (2d)	111 (i)	118 (2d)	192 (i)	195 (2d)	209 (i)	

The average normal blood glutathione level for 114 rats was found to be 44 ± 5.4 mg. per cent. The initial values of rats used for irradiation and those of rats of suitable age used for dietary studies constituted the series of values from which the normal average was calculated.

Statistical analysis of the data from series III, IV, V, and VI (rats 25-58) on the basis of paired observation shows a significant rise in blood glutathione values during the first 48 to 72 hours ($t = 3.5$, $n = 13$). Series I and II (rats 1-24) were not included in this analysis because the irradiation dose may have been slightly greater than in the remaining series due to scattering from the metal pan, and because earlier blood samples were taken which might have obscured the picture.

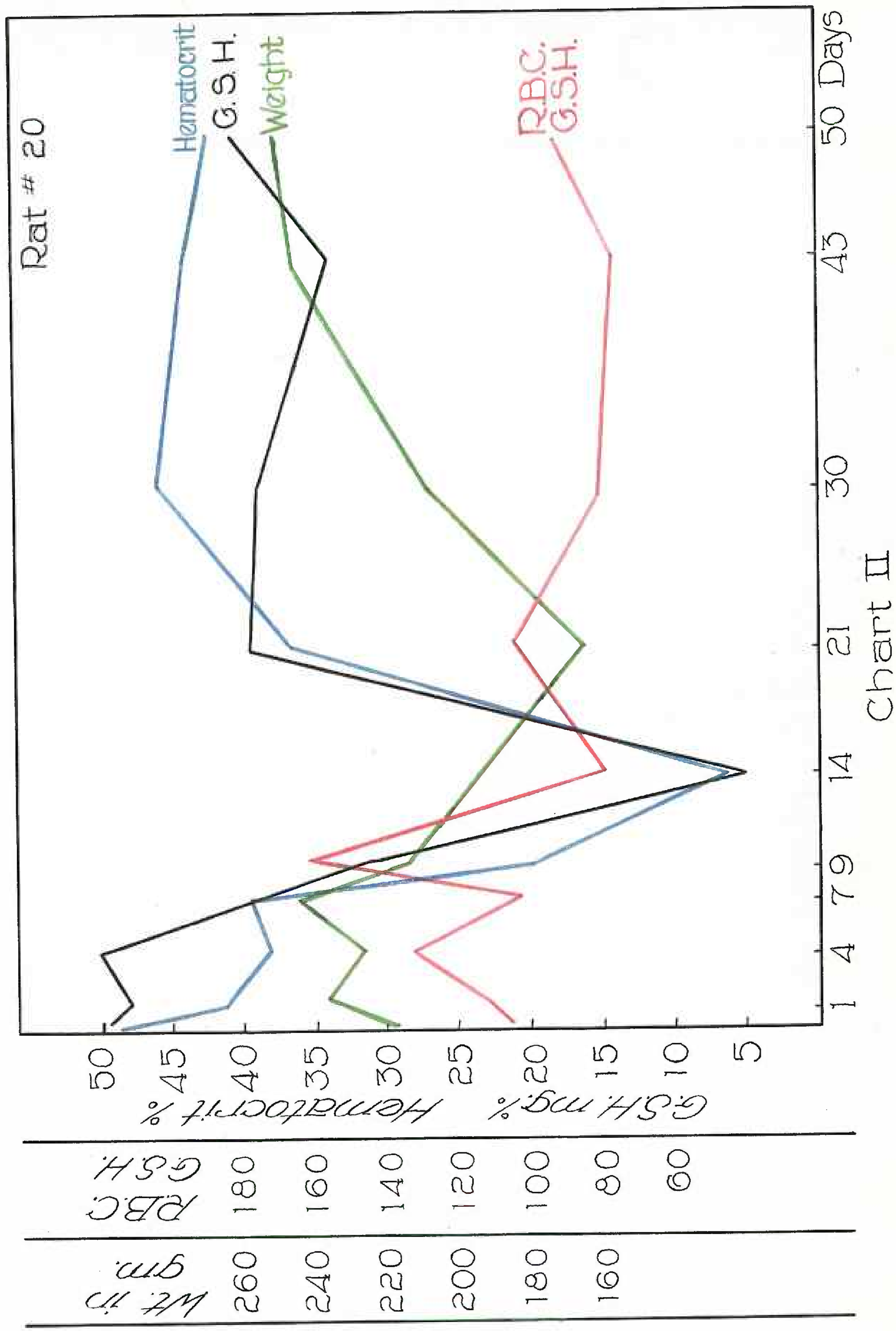
Of the 38 animals that survived for analysis between the sixth and nineteenth days, 30 showed blood glutathione levels lower than two times the standard deviation for the group of controls, and 22 fell below three times this standard deviation. The blood glutathione values of 6 animals fell to 10 mg. per cent or lower.

Hematocrit values generally decreased, sometimes very irregularly, after irradiation.

With the fall in hematocrit values and in total blood glutathione, the glutathione content of the red blood cells in some cases rose above the control range (as much or more than three times the standard deviation from the mean). The glutathione content of the red blood cells for a group of 68 animals averaged 107 ± 12 mg. per cent. In 9 animals on the eighth to the tenth days the hematocrit had fallen to below 21 and the red cell glutathione values had risen to 147 to 257 mg. per cent. In these cases the whole blood glutathione values generally were far below the normal range (8, 14, 15, 10, 18, 25, 31, 16, 19) as compared with the normal value of 44 mg. per cent.

When the total blood glutathione values dropped to very low levels, the glutathione content of the red blood cells in 5 animals fell to 77 mg. per cent or lower. In two of these cases liver biopsies were performed and samples of liver analyzed at the same time that blood glutathione determinations were run. In both instances the liver values were very low (89, 85, as compared with a normal liver glutathione of 184 mg. per cent). It appears that in these cases the liver was unable to supply adequate amounts of glutathione to the red blood cells.

Two rats (19 and 20) recovered after having very low blood glutathione levels and hematocrits. Chart II shows the values for rat number 20. On the fourteenth day the blood glutathione of this rat fell to 5 mg. per cent and the hematocrit to 6 per cent, after which the values rose gradually to control levels by the fiftieth day. The red cell glutathione rose and fell irregularly with variations in total blood glutathione and hematocrit values. It reached a peak value on the ninth day at which time both of the other values were falling rapidly. Minimal levels of red cell

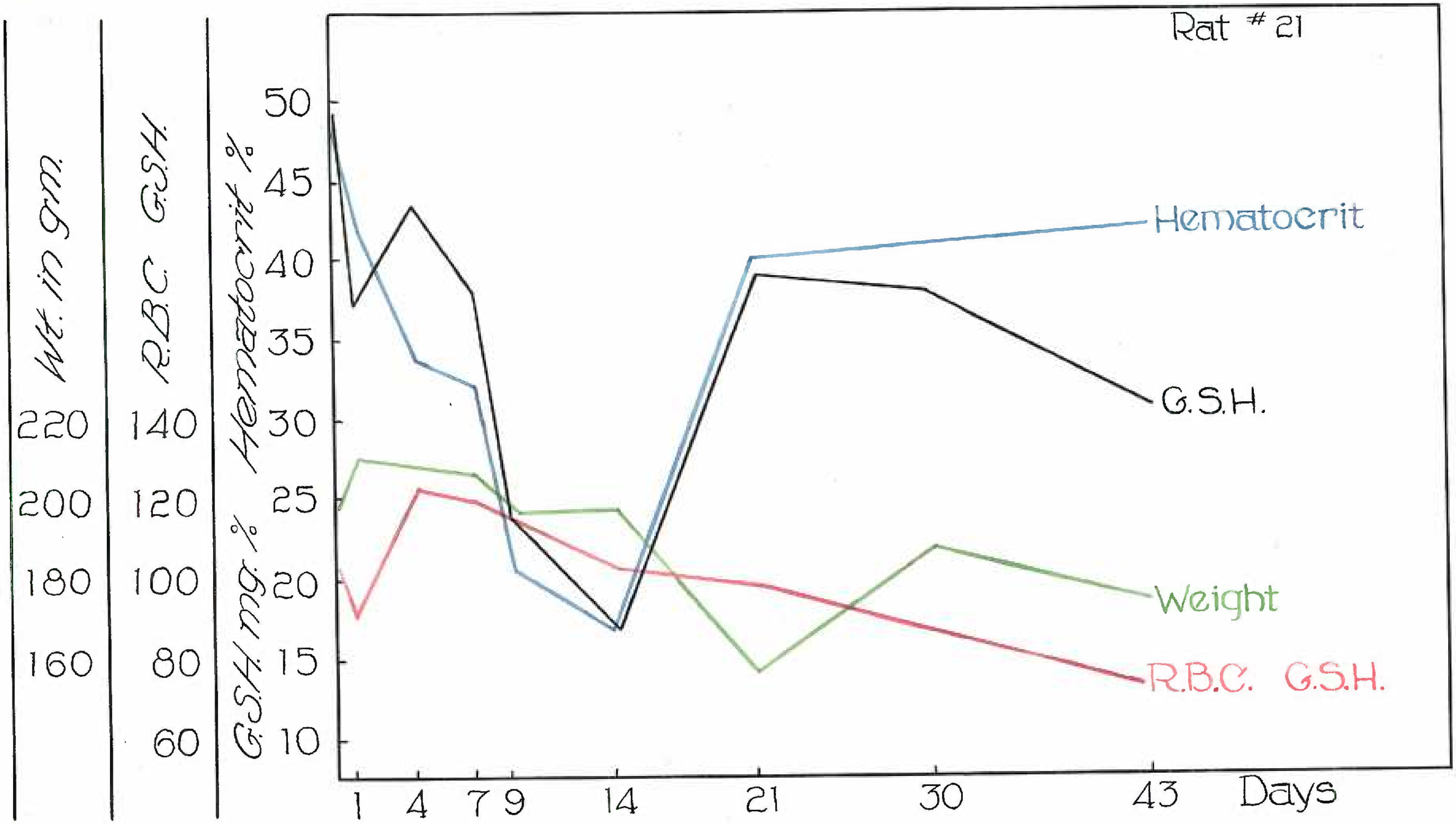

Rat # 20
Hematocrit
G.S.H.
Weight
R.B.C. G.S.H.
1 4 7 9 14 21 30 43 50 50 Days
Chart II
50 45 40 35 30 25 20 15 10 5
G.S.H. mg.% Hematocrit %
R.B.C. G.S.H.
180 160 140 120 100 80 60
Wt. in gm.
260 240 220 200 180 160

glutathione were reached on the fourteenth day when total blood glutathione and hematocrit values were very low, and on the forty-third day when the other values had risen toward the normal levels.

Chart III shows much the same picture for rat number 21 as for rat number 20 except that this animal succumbed following liver biopsy on the forty-third day, at which time its liver glutathione was low (85 mg. per cent).

Control values for liver glutathione on a total of 40 animals from series IV, V, and VI (rats 35 to 58) averaged 184 ± 20 mg. per cent. No low liver values were found before the eighth day, and in no case before the blood glutathione values had fallen to 31 mg. per cent or lower. Six low liver glutathione values were found and four of these were in rats with blood glutathione levels of 10 mg. per cent or lower. The liver biopsy on rat number 21 (blood 31 mg. per cent) was done on the thirtieth day after the animal had recovered from a blood glutathione level of 17 on the fourteenth day.

The above data show that the blood glutathione is generally lowered, and that the liver glutathione in some instances decreases when rats are subjected to a total body irradiation of 500 r. Since glutathione presumably functions in tissues to maintain enzymes in their active —SH forms it might be reasonable to assume that lowering of glutathione levels could place essential enzyme systems in jeopardy and interfere with vital body metabolism. This might account for the protective action of cysteine and



Rat # 21
Wt. in gm.
220
200
180
160
R.B.C. G.S.H.
140
120
100
80
60
G.S.H. mg. % Hematocrit %
50
45
40
35
30
25
20
15
10
1
4
7
9
14
21
30
43
Days
Hematocrit
G.S.H.
Weight
R.B.C. G.S.H.

Chart III

glutathione against the toxic effects of irradiation.

The relationship of glutathione to the red blood cell is not well understood. Literczek and Dinischiotu[89] have shown that glutathione readily concentrates in the red blood cells in vitro. They added glutathione to blood and found that within 2 hours the plasma was almost free of glutathione, while the concentration in the red cells had risen proportionately. It is also possible to wash red blood cells in saline repeatedly without loss of glutathione.

It has been reported[31, 40] that in both pernicious and secondary anemia the concentration of glutathione in the red blood cells rises as the hematocrit falls and returns to normal levels as the anemia is relieved.

Since we have found that the red blood cells in irradiated rats can store amounts of glutathione above normal levels at times when total blood glutathione is low, it appears that the red cells are able to maintain their glutathione concentration at normal or elevated levels under adverse circumstances.

However, Cronkite[85] reported in his talk before the Federated Societies (Atlantic City, April 17-22, 1950) that mice previously given glutathione showed less anemia after irradiation than did untreated animals. There was also earlier and greater hyperplasia of the spleen after irradiation in the animals receiving glutathione, and the bone marrow changes in these animals were different from those in animals not receiving glutathione. Thus it appears that glutathione deficiency may contribute to the anemia seen after irradiation.

SUMMARY

The studies on glutathione described in this thesis may be summarized as follows:

1. A modification of the method of Brückmann and Wertheimer[35] for the determination of glutathione in blood and liver has been described.

2. Data has been presented showing that the glutathione levels in rats vary with age from an average of 11 mgs. per cent at 7 days to 49 mgs. per cent in rats weighing over 190 gms.

3. It has been shown that it is very difficult to obtain low blood glutathione values in rats by variations in diet. Low protein diets containing casein caused a slight initial rise in blood glutathione levels. When the animals were changed to a low protein diet containing arachin (sulfhydryl deficient protein) the glutathione gradually fell to control levels and remained there until the experiment was terminated on the ninety-third day.

On Griffiths',[86] sulfhydryl deficient diet the blood glutathione levels showed a decrease after two months. The rats were in poor condition, only one surviving until the one hundred nineteenth day, when its blood glutathione value was 18 mg. per cent.

Rats placed on a protein-free diet maintained their blood glutathione levels at nearly control values for 37 days at which time they had lost about one-half of their original body weight.

A normal diet supplemented with 5 per cent ascorbic acid produced no change in blood glutathione levels.

The use of 10-20 per cent of cation exchange resins in a low sodium diet produced a fall in blood glutathione levels in 7 weeks, but left the animals in generally poor condition.

4. A study has been made of blood and liver glutathione values after 500 r total body X-irradiation. The data show that irradiation generally causes a decrease in blood glutathione levels and in some cases a lowering of liver glutathione values. The hematocrit generally falls simultaneously with total blood glutathione.

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