determined in the cytosol of all 4 cell strains. The results are given in the table. The Cd-resistant cell strains had about the same content of GSH as the 'wild-type' line. Consequently, induction of GSH did not explain the observed radioprotection. The concentration of total SH was increased 2.5 times for the HE_{100} cells and 4.2 times for the Cl $1D_{100}$ compared to the corresponding HE and Cl 1D cells. However, the amount of MT calculated on the basis of the metal content, accounted for the increase in SH groups in the Cd-resistant strains.

Flow cytometric DNA measurements in non-irradiated cells showed only small differences of the cell cycle phase distribution between the 'wild-type' cell lines and their corresponding MT-containing substrains during periods of exponential growth. The Cl 1D cells showed 62.7% of the cells in G_1, 21.8% in S and 15.5% in G_2. For Cl $1D_{100}$ the phase distribution was 59.8% (G_1), 20.1% (S), 20.0% (G_2); and for HE and HE_{100} cells 56.0% (G_1), 26.2% (S), 17.8% (G_2) and 58.5% (G_1), 23.2% (S), 18.2% (G_2), respectively. Thus, the radio-resistance observed apparently did not result from the accumulation of MT-containing cells in a radio-resistant phase.

Our results show that the murine Cl $1D_{100}$ cell line, with high intracellular levels of MT, has increased resistance against ionizing radiation. Growth curves for the clearly different human HE_{100} cell line indicate that these cells have also acquired resistance. MT accounts for the 3-4-fold increase of SH-groups in these cells compared to the corresponding non-resistant lines. Thus, for the 1st time it has been shown that radioprotection apparently can be provided by an endogenously produced protein, namely the extremely cysteine-rich metallothionein.

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2 R.W. Chen, D.J. Eakin and P.D. Whanger, Nutr. Rep. int. *10,* 195 (1971).
3 R.H.O. Bühler and J.H.R. Kägi, FEBS Lett. *39,* 229 (1974).
4 R. Mason, A. Bakka, G.P. Samarawickrama and M. Webb, Br. J. Nutr. *45,* 375 (1980).
5 A. Bakka and M. Webb, Biochem. Pharmac. *30,* 721 (1981).
6 M. Piscator, Nord. hyg. Tidskr. *48,* 76 (1964).
7 G.F. Nordberg, Envir. Physiol. *1,* 171 (1971).
8 J.K. Piotrowski, B. Trojanowska, J.M. Wisniewska-Knypl and W. Balanowska, in: Mercury, Mercurials and Mercaptans, p.247. Eds M.W. Miller and T.W. Clarkson. Charles C. Thomas, Springfield 1973.
9 S.Z. Cagen and C.D. Klaassen, Toxic. appl. Pharmac. *51,* 107 (1979).
10 A. Bakka, L. Endresen, A.B.S. Johnsen, P.D. Edminson and H.E. Rugstad, Toxic appl. Pharmac. *61,* 215 (1981).
11 Z.M. Bacq, in: Chemical protection against ionizing radiation, p.16. Charles C. Thomas, Springfield 1975.
12 H.E. Rugstad and T. Norseth, Nature *257,* 136 (1975).
13 H.E. Rugstad and T. Norseth, Biochem. Pharmac. *27,* 647 (1978).
14 A. Bakka and H.E. Rugstad, Acta pharmac. tox. *48,* 81 (1981).
15 L. Endresen, A. Bakka and H.E. Rugstad, Proc. 12th int. Congr. Chemother. Florence, Italy 1981, in press (1981).
16 G.L. Ellman, Archs Biochem. Biophys. *82,* 70 (1959).
17 F. Tietze, Analyt. Biochem. *27,* 502 (1969).
18 W. Göhde and W. Dittrich, Histochem. Ultrastrukt. suppl. *10,* 429 (1971).
19 E. Berkhan, Ärztl. Lab. *18,* 77 (1972).

Cysteine-induced effect on amino acids in neonatal rat brain

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Summary. In neonatal rat brain 6 h after s.c. administration of L-cysteine, an increase was observed in most of the amino acids with the exception of glutamic acid, aspartic acid, phenylalanine, sarcosine, glutamine, hydroxyproline and phosphoethanolamine compared to age-matched saline controls. Cysteine was not present at detectable levels in control brain but was found to be 0.38 to 0.52 µmole/g of fresh brain tissue in 2- and 4-day-old rats respectively after cysteine treatment.

Certain amino acids like glutamic acid, aspartic acid, cysteine sulfinic acid, cysteic acid and cysteine produced lesion in the brain when administered s.c. or p.o.[2,3]. Lesions induced by these compounds, with the exception of cysteine, are confined to specific regions of brain, such as the arcuate hypothalamic nucleus. Cysteine induces much more widespread lesions than the more acidic amino acids such as glutamic acid. Olney et al.[4] have postulated that cysteine-induced brain damage may be the result of conversion of cysteine to cysteine sulfinic acid and cysteic acid, which have been classified as neuroexcitatory amino acids by Curtis and his associates[5]. Efforts to produce a cysteine-type of lesion in 12-21-day-old mice have met with little success[4], despite that, the enzyme cysteine oxidase, which is responsible for the conversion of cysteine to cysteine sulfinic acid[6], was found at a higher level in that age group than in neonatal rats (1-4 days old), which is a vulnerable age for such damage. The present study was undertaken to determine whether cysteine enters the brain when injected s.c. and how it would affect the pool of other amino acids[7].

Methods. Pregnant Wistar strain rats in late gestation (18-20 days) were bought from Charles River Colony at Willmington in Massachusetts, and litters were delivered in our facilities. 2 and 4-day-old neonatal rats were used because earlier studies showed that young infants are more susceptible to cysteine-induced brain lesions than older rats. The dosage (1.2 mg/g) of L-cysteine was chosen on the basis of earlier studies[3], which showed that this dose produces widespread lesions in the central nervous system (CNS), with minimal mortality.

2 groups of rats (ages 2 and 4 days) were injected s.c. with a solution of L-cysteine in physiological saline (1.2 mg/g, neutralized to pH 7.0 with 1 N NaOH). They were decapitated 6 h later which was a sufficient time to produce maximum lesions[3]. Brains were removed and chilled in ice; immediately a pool of 6 brains from each group were weighed and homogenized in ice-cold 6% perchloric acid[8], in a ratio of 1 g fresh tissue to 5 ml of perchloric acid. The homogenate was centrifuged at 0 °C and the supernatant collected separately. The resultant pellet was washed twice by resuspending it in perchloric acid. The washing solution and supernatant were mixed and titrated to pH 4.0 with 2 N

Table 1. Amino acids concentration in brain of 2-day-old rats

	Normal	Cysteine-treated	Difference	Percentage
Phosphoserine	0.11	0.18	+0.07	63.6
Taurine	7.70	7.28	−0.42	5.4
Phosphoethanolamine	4.99	3.96	−1.03	20.6
Cysteic acid	0.15	0.17	+0.02	13.3
Urea	1.90	2.86	+0.96	50.5
Hydroxyproline	0.19	0.22	+0.03	15.8
±Allo-cystathionine	0.02	0.02	0	0
Glutamine	1.74	1.39	−0.35	20.0
Sarcosine	0.76	0.28	−0.48	63.0
GABA	1.07	1.21	+0.14	13.0
Ornithine	0.06	0.18	+0.12	200.0
Aspartic acid	1.52	1.54	+0.02	1.3
Threonine	0.45	0.73	+0.28	62.2
Serine	0.71	0.97	+0.26	36.6
Glutamic acid	4.55	3.35	−1.20	26.4
Proline	0.14	0.51	+0.31	264.3
Glycine	1.24	1.51	+0.27	21.8
Alanine	1.07	2.75	+1.68	157.0
Valine	0.09	0.56	+0.47	522.0
1/2 Cystine	0.00	0.38	–	–
Methionine	0.10	0.12	+0.02	20.0
Isoleucine	0.04	0.21	+0.17	425.0
Leucine	0.09	0.47	+0.38	422.0
Tyrosine	0.07	0.46	+0.39	557.1
Phenylalanine	0.05	0.03	−0.02	40.0
Lysine	0.16	0.46	+0.30	187.5
Histidine	0.10	0.34	+0.24	240.0
Arginine	0.04	0.08	+0.04	100.0

The results are the average of 2 measurements of 2 pools of rat brains. Average variation was about 5%. Values are expressed as μmoles amino acid per g of fresh tissue.

Table 2. Amino acids concentration in brain of 4-day-old rats

	Normal	Cysteine-treated	Difference	Percentage
Phosphoserine	0.06	0.14	+0.08	133.3
Taurine	4.79	5.98	+1.19	24.8
Phosphoethanolamine	4.67	3.40	−1.27	27.2
Cysteic acid	0.04	0.12	+0.08	200.0
Urea	0.73	1.37	+0.64	87.7
Hydroxyproline	0.12	0.07	−0.07	41.6
±Allo-cystathionine	0.01	0.07	−0.06	600.0
Glutamine	1.32	0.72	−0.60	45.4
Sarcosine	0.61	–	–	–
GABA	0.91	1.46	+0.55	60.4
Orinithine	0.05	0.13	+0.08	160.0
Aspartic acid	1.42	0.92	−0.50	35.2
Threonine	0.38	0.57	+0.19	50.0
Serine	0.54	0.70	+0.16	29.6
Glutamic acid	4.57	4.02	−0.55	12.0
Proline	0.12	0.47	+0.35	291.6
Glycine	0.99	1.21	+0.22	22.2
Alanine	0.65	2.52	+1.87	287.7
Valine	0.05	0.33	+0.28	560.0
1/2 Cystine	0.00	0.52	–	–
Methionine	0.09	0.15	+0.06	66.6
Isoleucine	0.02	0.17	+0.15	750.0
Leucine	0.05	0.42	+0.37	740.0
Tyrosine	0.11	0.42	+0.31	281.8
Phenylalanine	0.03	0.09	+0.06	200.0
Lysine	0.11	0.36	+0.25	227.0
Histidine	0.04	0.16	+0.12	300.0
Arginine	0.03	0.12	+0.09	300.0

The results are the average of 2 measurements of 2 pools of rat brains. Average variation was about 5%. Values are expressed as μmoles amino acids per g of fresh tissue.

KOH to precipitate potassium perchlorate. The suspension was kept in a cold room (0–5 °C) overnight. The next day, the whole suspension was centrifuged, the supernatant (extract) was lyophilized and dissolved in 0.2 M sodium citrate buffer (pH 2.2) representing 1.0 ml of buffer equivalent to 1.0 g of fresh brain tissue and frozen at −70 °C until used for amino acid analysis.

An aliquot of the extract equivalent to 50 mg of tissue was used for amino acid analysis. 2 separate aliquots were analyzed in each sample using the method reported by Feigin et al.[9]. Our data represent an average of 2 pools of rat brain from each age group. In both age groups, the control animals were injected with an equal volume of physiological saline instead of L-cysteine.

Results and discussion. It was observed (table 1) that in 2-day-old rats, glutamic acid, phenylalanine, sarcosine, glutamine, and phosphoethanolamine were decreased, whereas hydroxyproline, cysteic acid, taurine and ± allocystathionine showed no change in concentration. The other amino acids in L-cysteine-administered group were shown to increase 20–50% compared to the control saline-treated group.

In 4-day-old rats treated with L-cysteine (table 2), the amino acid concentrations were increased 25–750%. Cysteine-related amino acids, such as cysteic acid, taurine and ± allo-cystathionine were increased as well. The concentration of hydroxyproline in 4-day-old animals was decreased about 40% in comparison to the control group; it was unchanged in the 2-day-old rats. Amino acids whose concentration more than doubled are, proline, alanine, valine, isoleucine, leucine, tyrosine, lysine, histidine, and arginine. Taurine did not change in the 2-day-old rats but rose by about 25% in the 4-day-old rats. For phenylalanine, a 40% reduction was observed in the 2-day-old rats, whereas the 4-day-old rats had a 200% increase. The amount of cysteine in the cysteine-treated rats was 40% higher in 4-day-old than in 2-day-old rats, although in both age groups the dosages of injected cysteine had been the same.

It is evident from this study that concentration of most amino acids increased as a result of the cysteine-induced shift in the amino acid pool. More research is needed to determine whether this shift is due to transport from blood to brain or due to inhibition of the efflux of these amino acids from the brain[10]. However, the present study indicates that cysteine entered the brain and that a large proportion remained as cysteine was confirmed spectrophotometrically using Gaitonde's method[11]. Therefore, cysteine-induced neurotoxicity in infant rat brain may be due to cysteine itself rather than its acidic metabolite as previously was proposed by Olney et al.[4]. However, the cause of brain damage due to general imbalancing of other amino acid pools, at present cannot be ruled out.

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2 J.W. Olney and O.L. Ho, Nature *227*, 609 (1970).
3 J.W. Olney, O.L. Ho and V. Rhee, Exp. Brain Res. *14*, 61 (1971).
4 J.W. Olney, O.L. Ho, V. Rhee and B. Schainker, Brain Res. *45*, 309 (1972).
5 D.R. Curtis and J.M. Crawford, A. Rev. Pharmac. *9*, 209 (1969).
6 C.H. Misra and J.W. Olney, Brain Res. *97*, 117 (1975).
7 Y.P. Liu, L.M. Ambani and M.H. Van Woert, J. Neurochem. *19*, 2237 (1972).
8 H.C. Agrawal, J.M. Davis, E.E. Haynes and W.A. Himwitch, Ann. N.Y. Acad. Sci. *166*, 225 (1969).
9 R.D. Feigin, A.S. Klainer and W.R. Beisel, Metabolism *17*, 764 (1968).
10 A. Lajtha and J. Toth, J. Neurochem. *10*, 909 (1963).
11 M.K. Gaitonde, Biochem. J. *104*, 627 (1967).