Neurochemical Research (1) 53–63 (1976)

THE EFFECT OF METHIONINE ON THE UPTAKE, DISTRIBUTION, AND BINDING OF THE CONVULSANT METHIONINE SULFOXIMINE IN THE RAT

ROBERT A. SCHATZ, RONALD HARRIS, and OTTO Z. SELLINGER
Laboratory of Neurochemistry
Mental Health Research Institute
University of Michigan Medical Center
Ann Arbor, Michigan 48014

Accepted November 6, 1975

The effect of methionine on the uptake, distribution, and binding of the convulsant methionine sulfoximine (MSO) in 7 rat brain regions, the spinal cord, the liver, and the kidney was investigated. The administration of methionine decreased the uptake of MSO in all brain regions. The uptake of MSO by and its distribution in the nervous tissue was uniform and failed to result in any preferential accumulation of the drug. Methionine decreased the amount of MSO bound to cerebral structures and to the spinal cord. MSO bound to the spinal cord was less susceptible to release by Triton X-100 than was brain-bound MSO.

INTRODUCTION

For several years, various approaches have been used in an attempt to elucidate the mechanism of action of the convulsant L-methionine-*dl*-sulfoximine (MSO). These have included studies on the effect of MSO on the cerebral metabolism of amino acids and ammonia (1–8), brain amines (9, 10), glycogen (7, 11), protein (12–14), and cerebral ultrastructure (8, 15, 16). The antagonism of MSO seizures by methionine is also well documented (3, 17, 18). There is evidence for methionine antagonism of the effect of MSO on "bound" acetylcholine production in rat

TABLE I
THE EFFECT OF PERFUSION ON TISSUE RADIOACTIVITY LEVELS[a]

Tissue	dpm/g	
	Buffer + MSO	Buffer Only
Brain	36,550	36,866
Spinal Cord	27,150	26,460
Liver	179,250	164,969
Kidney	298,950	278,315

[a] [^{3}H]MSO was injected 3 hr prior to sacrifice. The total radioactivity values are means obtained from 2 (buffer + MSO) and 4 (buffer only) animals. For perfusion details, see Experimental Procedure.

cortical slices (19), rat brain *S*-adenosyl-L-methionine levels (20), mouse cerebellar ammonia (7), and rat brain glutamine synthetase (3). MSO and methionine have also been shown to be uptake antagonists (21, 22).

We have investigated the effect of methionine on the uptake, distribution and binding of [^{3}H]MSO in 7 brain regions, the spinal cord, the liver, and the kidney of the adult rat. We also attempted to establish whether methionine antagonizes MSO by disallowing MSO to reach its nervous tissue targets in sufficiently high amounts to elicit seizures.

EXPERIMENTAL PROCEDURE

Animals and Drug Treatment. Adult male Sprague–Dawley rats weighing 100–160 g were used throughout the study. A mixture of nonradioactive (Sigma Chemical Co., St. Louis, Missouri) and radioactive MSO (0.94 mmol/kg) was administered intraperitoneally alone or jointly with L-methionine (4.7 mmol/kg) (ICN Biochemicals, Cleveland, Ohio) in a total volume of 10 ml/kg in saline. The animals were sacrificed 1, 3, or 6 hr later, i.e., well before (1 and 3 hr) the onset of the seizure, or immediately preceding it (6 hr).

Purification of [^{3}H]MSO. Methyl tritiated MSO (5.1 mCi/mmol), purified as previously described (23), was chromatographed on cellulose thin-layer-chromatography (TLC) plates (EM Laboratories, Elmsford, New York) in *n*-butanol, glacial acetic acid, and water (2:1:1), where it migrated identically with authentic nonradioactive MSO. Its final specific radioactivity was 3.67 mCi/mmol. Solutions of [^{3}H]MSO were diluted for injection with nonradioactive MSO to achieve specific radioactivities of about 0.4 mCi/mmol.

Preparation of Tissue. To minimize the contribution of circulating [^{3}H]MSO to the estimation of the tissue radioactivity, animals were perfused immediately prior to sacrifice with either ice-cold 0.1 M sodium phosphate buffer, pH 7.4, or buffer containing 17 mg/liter of nonradioactive MSO. As shown in Table I, identical results were achieved,

indicating that the presence of MSO in the perfusing medium had no effect on the measurement of the tissue stores of [^{3}H]MSO. Perfusion was for 5 min, i.e., until the perfusate was totally discolored. After perfusion, the brains were removed and dissected (24) into cerebellum (CL), brainstem (BS), striatum (ST), hypothalamus (HY), midbrain (MB), hippocampus (HI), and cerebral cortex (CX). The spinal cord, liver, and kidneys were also removed and, along with the brain regions, were weighed and homogenized in 10 vol of ice-cold 0.32 M sucrose. The homogenates were centrifuged at 100,000 *g* for 70 min. The resulting pellet was washed with an equal volume of sucrose and the suspension was centrifuged as above yielding the "sucrose supernatant." The washed "sucrose pellet" was homogenized in an equal volume of Triton X-100 (0.02%) (w/v) (Sigma Chemical Co.) and was centrifuged as above yielding a "Triton supernatant" and a "Triton pellet" which was homogenized in ice-cold deionized water.

Determination of Radioactivity. Aliquots of the whole homogenate, the "sucrose supernatant," the "Triton supernatant," and of the final "Triton pellet" suspension were digested in 1 ml of 2 N NaOH at 65°C for 16 hr. Samples were neutralized with 0.2 ml of 12 N HCl, and 10 ml PCS (Amersham Searle, Arlington Heights, Illinois) was added for determination of radioactivity.

Protein Determination. Protein was determined by the Lowry procedure (25).

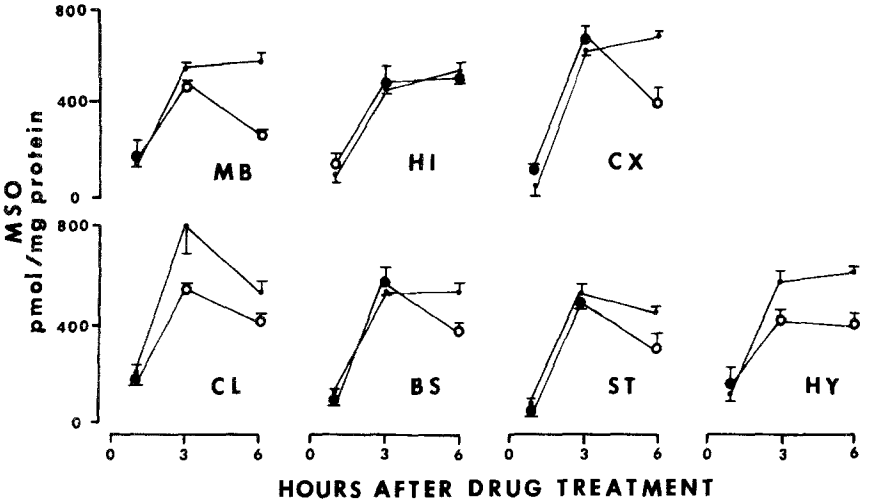

FIG. 1. The effect of methionine on the regional uptake of [^{3}H]MSO in rat brain. •——•: [^{3}H]MSO; ●——● [^{3}H]MSO + methionine. For dosages, see Experimental Procedure. Each point is the mean ± SEM obtained from the number of animals indicated by the numeral in parentheses in CX, which is the same for all regions. The values represent pmoles of [^{3}H]MSO/mg of protein in the regional homogenate. Open circles, ○——○, denote a significant difference at the 0.05 level (see Experimental Procedure) from rats receiving only [^{3}H]MSO. MB: midbrain; HI: hippocampus; CX: cortex; CL: cerebellum; BS: brainstem; ST: striatum; HY: hypothalamus.

Statistical Analyses. Statistical comparison of MSO vs. MSO + methionine-treated groups was done using the two-tailed Student's *t* test.

RESULTS

Uptake of [^{3}H]MSO by Tissues

There were no significant differences between MSO and MSO + methionine-treated rats in any of the brain regions at 1 hr (Figure 1). At 3 hr, the midbrain, the cerebellum, and the hypothalamus of animals receiving MSO + methionine contained significantly less [^{3}H]MSO than was found in animals receiving MSO alone. At 6 hr [^{3}H]MSO levels in all brain regions, except the hippocampus, were lower than at 3 hr. The levels of [^{3}H]MSO were highest in the cerebellum at 3 hr (about 800 pmol/mg of protein) (Figure 1) while in all other brain regions they failed to exceed 700 pmol/mg of protein. The levels of [^{3}H]MSO were

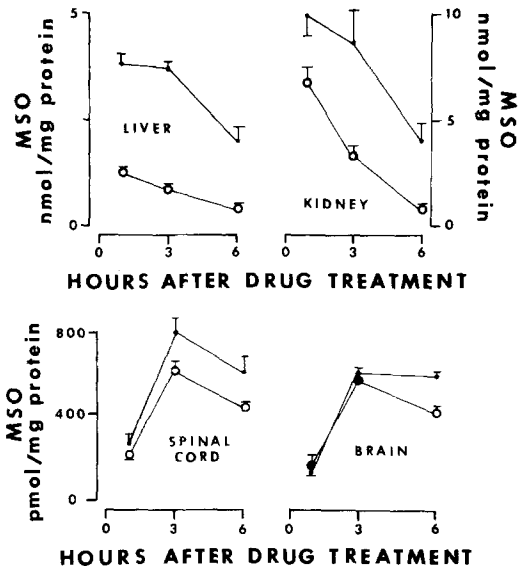

FIG. 2. The effect of methionine on the uptake of [^{3}H]MSO by whole brain, spinal cord, liver, and kidney. •——•: [^{3}H]MSO; ●——●: [^{3}H]MSO + methionine. For dosages, see Experimental Procedure. Each point is the mean ± SEM obtained from the number of animals indicated by the numeral in parentheses in kidney which is the same for all organs. The values represent pmoles or nmoles of [^{3}H]MSO/mg of protein in the organ homogenate. Open circles, ○——○, denote a significant difference at the 0.05 level (see Experimental Procedure) from rats receiving only [^{3}H]MSO.

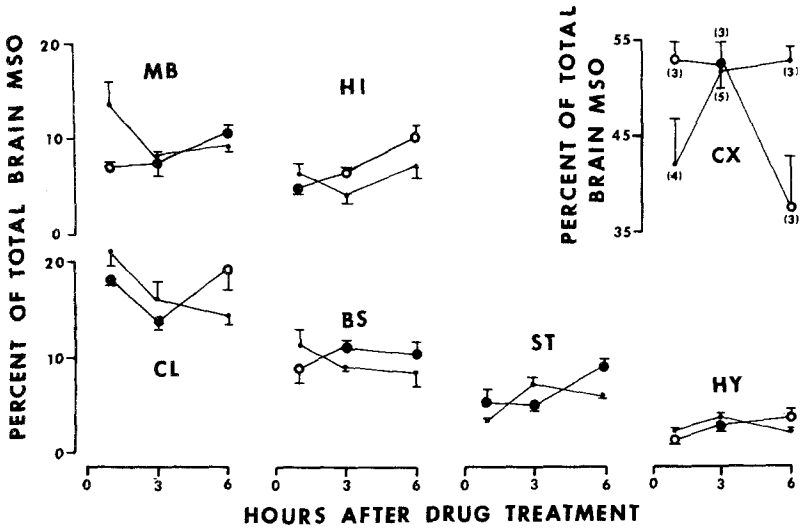

FIG. 3. Effect of methionine on the regional distribution of MSO in rat brain. •——•: [^{3}H]MSO; ●——●: [^{3}H]MSO + methionine. For dosages, see Experimental Procedure. For abbreviations, see legend to Figure 1. Each point represents the mean ± SEM obtained from the number of animals indicated by the numeral in parentheses in CX, which is the same for all regions. The values refer to [^{3}H]MSO present in the region as a percent of the radioactivity in the whole brain homogenate. As in Figure 1, open circles, ○——○, denote a significant difference at the 0.05 level (see Experimental Procedure) from rats receiving only [^{3}H]MSO. Note difference in y-axis scales between CX and all other regions.

significantly reduced by the administration of methionine, particularly in the spinal cord and the peripheral organs (Figure 2). This methionine-induced decrease became significant in the whole brain only at 6 hr (Figure 2). The levels of [^{3}H]MSO in the spinal cord reached the cerebellar values by 3 hr and thus exceeded those of any other brain region. During the 6-hr experimental period the levels of [^{3}H]MSO in the peripheral organs decreased from 10 to 4 nmol/mg of protein in the kidney and from 4 to 2 nmol/mg of protein in the liver (Figure 2).

Regional Distribution of [^{3}H]MSO in Rat Brain

Only the cortex showed a sharp increase in its share of the total tissue [^{3}H]MSO between 1 and 3 hr; in all other regions the 3 hr values were not apparently different from those at 1 hr. One hour after the administration of [^{3}H]MSO + methionine, there was less [^{3}H]MSO in all brain regions, except in the cortex and the striatum, than in the corresponding regions of animals which received [^{3}H]MSO only (Figure 3). At 3 hr,

except for the hippocampus which contained a greater share of the total [^{3}H]MSO in animals receiving [^{3}H]MSO + methionine than in those receiving [^{3}H]MSO alone, there was no significant difference in the percentages of [^{3}H]MSO between the two groups of animals. Finally, at 6 hr a higher percentage of the total [^{3}H]MSO was found in the hippocampus, the cerebellum, and the hypothalamus of animals receiving [^{3}H]MSO + methionine than in those receiving [^{3}H]MSO only; conversely a lower percentage was found in the cortex of animals receiving [^{3}H]MSO + methionine than was present in those receiving [^{3}H]MSO only.

Effect of Methionine on [^{3}H]MSO Binding

"Bound" [^{3}H]MSO is defined in this paper as the [^{3}H]MSO recovered in the "sucrose pellet" (see Experimental Procedure). The amount of bound [^{3}H]MSO was calculated by combining the values found in the "Triton supernatant" and the "Triton pellet" and is

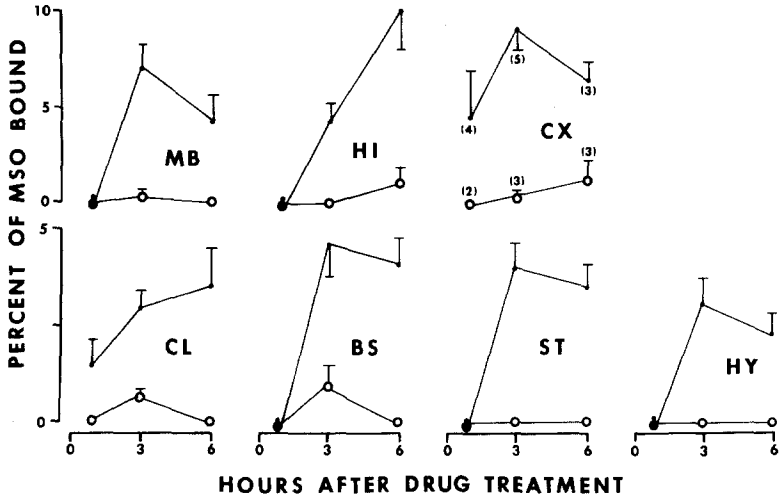

FIG. 4. The effect of methionine on the regional binding of [^{3}H]MSO in rat brain. •——•: [^{3}H]MSO; ●——●: [^{3}H]MSO ± methionine. For dosages, see Experimental Procedure. For abbreviations, see the legend to Figure 1. Each point represents the mean ± SEM obtained from the number of animals indicated by the numeral in parentheses in CX, which is the same for all regions. The values refer to [^{3}H]MSO recovered in the washed "sucrose pellet" (see Experimental Procedure) as percent of the radioactivity present in the regional homogenate. As in Figures 1 and 3, open circles, O——O, denote a significant difference at the 0.05 level (see Experimental Procedure) from rats receiving only [^{3}H]MSO.

TABLE II

THE EFFECT OF TRITON X-100 ON RELEASE OF [^{3}H]MSO IN RAT BRAIN REGIONS AFTER TREATMENT WITH MSO OR MSO + METHIONINE[a]

Hours after treatment	N	Treatment	Brain region						
			Cere bellum	Brainstem	Striatum	Hypo-thalamus	Midbrain	Hippo-campus	Cortex
1	3	MSO	100 (24)	—	—	—	—	—	100 (34)
	2	MSO + methionine	—	—	—	—	—	—	—
3	5	MSO	75 (123)	55 (148)	93 (117)	100 (82)	62 (165)	60 (137)	71 (264)
	3	MSO + methionine	32 (17)	0 (13)	—	—	0 (4)	—	0 (2)
6	3	MSO	100 (126)	70 (253)	82 (74)	66 (123)	50 (56)	88 (61)	80 (179)
	3	MSO + methionine	—	—	—	0	—	—	—

[a] Values refer to % of [^{3}H]MSO released by 0.02% Triton. Values in parentheses are pmol/mg of protein of [^{3}H]MSO bound and represent 100%. Dashed lines signify absence of any [^{3}H]MSO binding. For drug schedule and description of binding experiments see Experimental Procedure. N = number of animals.

expressed as the percentage of the total [^{3}H]MSO in the tissue homogenate. At 1 hr, only the cerebellum and the cortex contained any bound [^{3}H]MSO (Figure 4). Highest values of bound [^{3}H]MSO were found in the hippocampus at 6 hr and in the cortex at 3 hr (about 9%). A notable finding, shown in Figure 4 as well as in Table II, was that methionine greatly reduced the binding of [^{3}H]MSO in all brain regions. This finding could be confirmed when bound [^{3}H]MSO determinations were performed on samples of whole brain (data not shown).

Effect of Triton X-100 on MSO Release

Triton X-100 homogenization (see Experimental Procedure) released all of the bound [^{3}H]MSO (Table II) at 1 hr, while at 3 and 6 hr this treatment proved effective only in releasing [^{3}H]MSO bound in the absence of methionine. The small amounts of [^{3}H]MSO bound when methionine was coadministered (Figure 4) became resistant to Triton except in the cerebellum, where 32% of the tissue-bound [^{3}H]MSO was released. Triton X-100 also effectively released [^{3}H]MSO bound in the peripheral organs, although this process appeared to diminish in intensity with time (Table III).

DISCUSSION

The concurrent administration of [^{3}H]MSO + methionine resulted, within 3–6 hr, in general and significant reductions of [^{3}H]MSO uptake by all brain regions except the hippocampus (Figure 1). This finding extends our previous observation (22) which dealt with the cortex and the cerebellum only, to 5 additional brain regions. Furthermore, as shown in Figure 2, significantly less [^{3}H]MSO was present in the spinal cord, the liver, and the kidney of animals receiving the combination of [^{3}H]MSO + methionine than in those receiving only [^{3}H]MSO. This protective effect of methionine became apparent as early as 1 hr postadministration and lasted throughout the entire preconvulsant period.

The accumulation of [^{3}H]MSO in the brain regions (Figure 3) was, in general, directly proportional to their weights. There was, however, a shift in the regional partition of [^{3}H]MSO with time, as evidenced by its marked increase, between 1 and 3 hr, in the cortex and its parallel decrease in the cerebellum, the midbrain, the brainstem, and the hippocampus. It is of particular interest that the administration of methionine altered the time course of the regional partition of [^{3}H]MSO

TABLE III
THE EFFECT OF TRITON X-100 ON RELEASE OF [^{3}H]MSO IN RAT ORGANS AFTER TREATMENT WITH MSO OR MSO + METHIONINE[a]

Hours after treatment	N	Treatment	Rat organ			
			Brain	Spinal cord	Liver	Kidney
1	3	MSO	100 (8)	100 (227)	100 (579)	96 (669)
	2	MSO + methionine	—	100 (27)	78 (122)	100 (688)
3	5	MSO	79 (148)	100 (105)	71 (158)	94 (720)
	3	MSO + methionine	14 (4)	—	0 (37)	87 (320)
6	3	MSO	81 (90)	44 (63)	40 (102)	39 (369)
		MSO + methionine	0 (2)	—	60 (35)	0 (15)

[a] Values refer to % of [^{3}H]MSO released by 0.02% Triton. Values in parentheses are pmol/mg of protein of [^{3}H]MSO bound and represent 100%. Dashed lines signify absence of any [^{3}H]MSO binding. For drug schedule and description of binding experiments see Experimental Procedure. N = number of animals.

for, under these conditions, the drug reached high cortical levels at 1 hr, but, by 3 hr, appeared to decay and accumulate instead in the cerebellum and the hippocampus.

Although quantitatively much inferior, the binding of [^{3}H]MSO in brain appeared tighter when the drug was given with methionine (Tables II and III), rather than alone, a phenomenon briefly noted before (22). An increase in Triton-resistant [^{3}H]MSO binding in the peripheral organs with time was also noted, and this occurred after administering [^{3}H]MSO by itself and with methionine (Table III). [^{35}S]MSO binding in mouse brain and liver was previously reported by Rao and Meister (26) who identified the presumably protein-bound radioactivity as MSO phosphate. We have heretofore failed to demonstrate soluble MSO metabolites in rat brain (22, 25). Moreover, more recently we conducted a number of chromatographic experiments using a variety of organ extracts of [^{3}H]MSO-treated rats and uncovered no evidence for the existence of soluble [^{3}H]MSO metabolites.

ACKNOWLEDGMENT

This work was supported by the USPHS (grants MH 07417 and NS 06294) and a grant from the Epilepsy Foundation of America.

REFERENCES

1. PETERS, E.L., and TOWER, D.B. 1959. Glutamic acid and glutamine metabolism in cerebral cortex after seizures induced by methionine sulfoximine. J. Neurochem. 5:80–90.
2. VAN DEN BERG, C.J., and VAN DEN VELDEN, J. 1970. The effect of methionine sulfoximine on the incorporation of labelled glucose, acetate, phenylalanine and proline into glutamate and related amino acids in the brains of mice. J. Neurochem. 17:985–991.
3. SELLINGER, O.Z., AZCURRA, J.M., and OHLSSON, W.G. 1968. Methionine sulfoximine seizures VIII. The dissociation of the convulsant and glutamine synthetase inhibitory effects. J. Pharmacol. Exp. Ther. 164:212–222.
4. TEWS, J.K., and STONE, W.E. 1964. Effects of methionine sulfoximine on levels of free amino acids and related substances in brain. Biochem. Pharmacol. 13:543–545.
5. GHITTONI, N.E., and SELLINGER, O.Z. 1970. Cerebral methionine and cysteine levels in rats after injection of the convulsant methionine sulfoximine. Pharmacol. Res. Commun. 2:117–120.
6. STRANSKY, Z. 1969. Time course of rat brain GABA levels following methionine sulfoximine treatment. Nature (London) 224:612–613.
7. FOLBERGROVA, J., PASSONNEAU, J.V., LOWRY, O.H., and SCHULZ, D.W. 1969. Glycogen, ammonia and related metabolites in the brain during seizures evoked by methionine sulfoximine. J. Neurochem. 16:191–203.
8. HINDFELT, B., and PLUM, F. 1975. L-methionine DL-sulfoximine and acute ammonia toxicity. J. Pharm. Pharmacol. 27:456–458.
9. DEROBERTIS, E., SELLINGER, O.Z., RODRIGUEZ DE LORES ARNAIZ, G., ALBERICI, M., and ZIEHER, L.M. 1967. Nerve endings in the methionine sulphoximine convulsant rats, a neurochemical and ultrastructural study. J. Neurochem. 14:81–89.
10. SCHATZ, R.A., and SELLINGER, O.Z. 1975. The elevation of cerebral histamine-*N*- and catechol-*O*-methyl transferase activities by L-methionine-*dl*-sulfoximine. J. Neurochem. 25:73–78.
11. PHELPS, C.H. 1975. An ultrastructural study of methionine sulphoximine-induced glycogen accumulation in astrocytes of the mouse cerebral cortex. J. Neurocytol. 4:479–490.
12. SELLINGER, O.Z., AZCURRA, J.M., OHLSSON, W.G., KOHL, H.H., and ZAND, R. 1972. Neurochemical correlates of drug-induced seizures: Selective inhibition of cerebral protein synthesis by methionine sulfoximine. Fed. Proc. 31:160–165.
13. FOLBERGROVA, J. 1962. Effect of D,L-methionine sulfoximine on incorporation of [^{35}S]-methionine into proteins of brain cortex slices. Nature 194:871–873.
14. LISY, V., and LODIN, Z. 1975. The influence of L-methionine-D,L-sulfoximine on the labelling of acid soluble fraction and proteins of the brain cortex after intraperitoneal injection of ^{14}C-leucine. Exp. Pathol. (Jena) 10:211–215.
15. RIZZUTO, N., and GONATAS, N.K. 1974. Ultrastructural study of the effect of methionine sulfoximine on developing and adult rat cerebral cortex. J. Neuropathol. Exp. Neurol. 33:237–251.
16. GUTIERREZ, J.A., and NORENBERG, M.D. 1975. Alzheimer II astrocytosis following methionine sulfoximine. Arch. Neurol. 32:123–126.
17. REINER, L., MISANI, F., and WEISS, P. 1950. Studies on nitrogen trichloride treated prolamines. VI. Suppression of the development of convulsions with methionine. Arch. Biochem. 25:447–454.

18. LODIN, Z., and KOLOUSEK, J. 1958. The effect of some amino acids on the neurotoxic action of methionine sulfoximine. Physiol. Bohemosl. 7:87–94.
19. TOWER, D.B., and ELLIOTT, K.A.C. 1953. Experimental production and control of an abnormality in acetylcholine metabolism present in epileptogenic cortex. J. Appl. Physiol. 5:375–391.
20. SCHATZ, R.A., and SELLINGER, O.Z. 1975. Effect of methionine and methionine sulfoximine on rat brain *S*-adenosyl methionine levels. J. Neurochem. 24:63–66.
21. ROTH, J.S., WASE, A., and REINER, L. 1952. The distribution of S^{35}-labeled L-methionine sulfoximine in the rat. Science 115:236–238.
22. GHITTONI, N.E., OHLSSON, W.G., and SELLINGER, O.Z. 1970. The effect of methionine on the regional and intracellular disposition of [^{3}H]-methionine sulfoximine in rat brain. J. Neurochem. 17:1057–1068.
23. BRINK, J.J., and SELLINGER, O.Z. 1967. A rapid, preparative separation of methionine sulfoximine from methionine sulfoxide. J. Chromatog. 27:269–270.
24. GLOWINSKI, J., and IVERSEN, L.L. 1966. Regional studies of catecholamines in the rat brain. I. The disposition of H^3 norepinephrine, H^3 dopamine and H^3 dopa in various regions of the brain. J. Neurochem. 13:655–669.
25. LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., and RANDALL, R.J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265–275.
26. RAO, S.L.N., and MEISTER, A. 1972. *In vivo* formation of methionine sulfoximine phosphate, a protein-bound metabolite of methionine sulfoximine. Biochem. 11:1123–1127.
27. LAMAR, C., JR., and SELLINGER, O.Z. 1965. The inhibition *in vivo* of cerebral glutamine synthetase and glutamine transferase by the convulsant methionine sulfoximine. Biochem. Pharmacol. 14:489–506.