After exposure to hydroxyurea, monolayers were rinsed with complete medium and harvested with 0.05 percent trypsin. The resulting single-cell suspension was counted, and appropriate numbers of cells were plated onto petri dishes.

At intervals after 0.5 hour at 37°C (to permit cell attachment), groups of three dishes were irradiated with 750 r. After irradiation at 1 hour, 6 hours, and 11 hours after synchrony, cells were incubated in EBSS for periods up to 6 hours. Cells were then re-fed with FS-15 for colony formation. There is increased response when cells are in the presynthetic (G_1, 0 to 2 hours), postsynthetic (G_2, 7 to 9 hours) and mitotic (M, 9 to 10 hours) phases, and decreased response during the DNA synthetic (S, 2 to 7 hours) phase (Fig. 2). The ability to repair potentially lethal damage is dependent on cell age. Cells which are in middle or late S appear to have substantial capacity for repair, but cells in early or late G_1 do not appear to have this capacity. Because repair of potentially lethal radiation damage is apparently accomplished most efficiently by cells in the process of replicating their DNA, it is not unreasonable to expect that repair mechanisms and the replicative process are associated.

Any environment which is not optimum with regard to division may provide a milieu which allows repair to be functionally effective. The following should provide such environments: (i) low temperature, (ii) hypoxia, and (iii) certain metabolic inhibitors. Whitmore and Gulyas (*3*) have shown survival increase in synchronous mouse L cells when placed at 5°C after irradiation; an effect similar to that reported here has been observed in late (hypoxic) P-388 ascites tumors when allowed to remain hypoxic after exposure (*8*).

Phillips and Tolmach (*5*) have reported survival enhancement in HeLa S3-3 cells exposed to cyclohexamide after irradiation. Therefore, we feel that EBSS provides an environment permitting repair while preventing or slowing progression toward the first division after irradiation.

JAMES A. BELLI*
MERIJEAN SHELTON
Section of Radiation Biology, Department of Radiology, University of Texas Southwestern Medical School, Dallas 75235

References and Notes

1. M. M. Elkind and H. Sutton, *Radiation Res.* **13**, 556 (1960); M. M. Elkind, H. Sutton-Gilbert, W. B. Moses, T. Alescio, R. W. Swain, *ibid.* **25**, 359 (1965); M. M. Elkind, G. F. Whitmore, T. Alescio, *Science* **143**, 1454 (1964); M. M. Elkind, W. B. Moses, H. Sutton-Gilbert, *Radiation Res.* **31**, 156 (1967).
2. R. Z. Lockart, Jr., M. M. Elkind, W. B. Moses, *J. Nat. Cancer Inst.* **27**, 1393 (1961); J. E. Till and E. A. McCulloch, *Radiation Res.* **18**, 96 (1963); J. A. Belli, G. J. Dicus, F. J. Bonte, *J. Nat. Cancer Inst.* **38**, 673 (1967); E. J. Hall and L. G. Lajtha, *Radiation Res.* **20**, 187 (1963); S. Bacchetti and F. Mauro, *ibid.* **25**, 103 (1965).
3. G. F. Whitmore and S. Gulyas, *Nat. Cancer Inst. Monogr.* **24**, 141 (1967).
4. F. Mauro and M. M. Elkind, *Science* **155**, 1561 (1967).
5. R. A. Phillips and L. J. Tolmach, *Radiation Res.* **29**, 413 (1966).
6. The parent line was provided by Dr. M. M. Elkind, NIH, Bethesda, Maryland.
7. J. A. Belli and F. J. Bonte, *Radiation Res.* **18**, 272 (1963).
8. G. J. Dicus, W. Nagle, J. A. Belli, in preparation.
9. Supported by PHS grants CA-08162 and 5T1-CA-5136 and American Cancer Society grant T-461. J.A.B. held PHS career development award, 1-K3-CA-16, 925 (1965-1968).
* Present address: Department of Radiology, Harvard Medical School, 50 Binney Street, Boston, Massachusetts 02115.

4 June 1969

Indole(ethyl)amine N-Methyltransferase in the Brain

Abstract. *An enzyme which* N*-methylates various indole(ethyl)amine substrates was isolated from brain, separated from the pituitary and the pineal glands. It appeared localized in the supernatant and synaptosomal areas after discontinuous sucrose-gradient ultracentrifugation. This is the first demonstration of the enzyme in brain.*

Axelrod (*1*) has described a nonspecific *N*-methyltransferase in lung adrenal, kidney, spleen, heart, and liver of rabbit, but not in the brain of rabbit or the lung of other species. We have now found a similar enzyme in the brain of the chick. Our enzyme appears, however, to be more specific than that found by Axelrod in that it fails to *N*-methylate phenylethylamine substrates.

Table 1. Substrate specificity of chick brain indole(ethyl)amine *N*-methyltransferase. Purified enzyme (10 μg of protein) from chick brain was incubated with 5 μmole of substrate and *S*-adenosylmethionine-methyl-^{14}C. After 90 minutes of incubation, the ^{14}C-methyl-labeled metabolites were isolated from the reaction mixture and the radioactivity measured.

Substrate	Relative activity (%)
5-Hydroxytryptamine	100
5-Methoxytryptamine	88
Tryptamine	60
N-Methyltryptamine	47
Norepinephrine	0
5-Hydroxy-*N*,*N*-dimethyltryptamine	0

White Leghorn cockerels (5 days old) were decapitated, and the whole brain was dissected free from the pituitary stalk and overhanging pineal. The brains were homogenized in five volumes of 0.32*M* sucrose. The enzyme content of whole-brain homogenates and supernatants from centrifugation at 100,000*g* in 0.25*M* phosphate buffer (*p*H 7.9) was estimated with 5-hydroxytryptamine (5HT) as a substrate and *S*-adenoxylmethionine–methyl-^{14}C as a methyl donor. This mixture was incubated for 90 minutes at 37°C. The reaction was stopped by the addition of borate buffer (*p*H 10); radioactive methylated products were extracted into water-saturated isoamyl alcohol and the radioactivity was determined in a liquid scintillation counter.

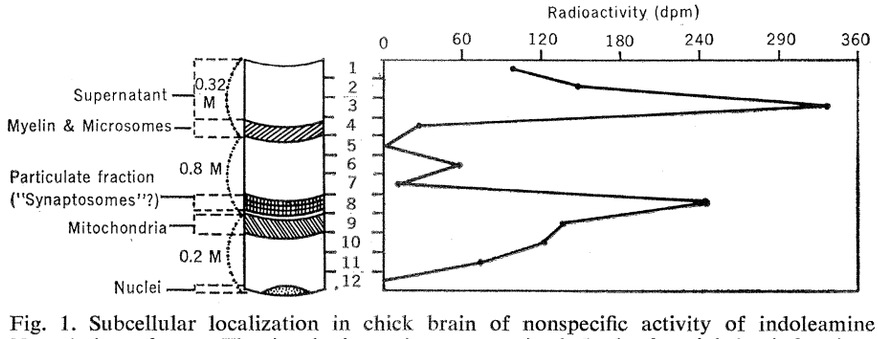

Fig. 1. Subcellular localization in chick brain of nonspecific activity of indoleamine *N*-methyltransferase. The incubation mixture contained 5 μl of serial 3-ml fractions from the sucrose gradient; 5-hydroxytryptamine was the substrate. The results are expressed in disintegrations per minute (dpm).

The specific activity (number of millimicromoles of *N*-methyl serotonin formed per milligram of protein in 90 minutes) of the supernatant fraction of whole-brain homogenate was 0.26. The enzyme from supernatant was purified by ammonium sulfate precipitation, with the highest specific activity (0.87) being found in the protein precipitated in the 45 to 55 percent ammonium sulfate fraction. This fraction was further purified on Sephadex G-100 equilibrated with 0.05*M* phosphate buffer (*p*H 7.5) with an approximate tenfold overall purification (specific activity, 2.54). Enzyme activity was linear with 5HT concentration (0 to 2.0 μmole). Boiled enzyme was inactive. The enzyme was localized in the subcellular fraction after discontinuous sucrose-gradient centrifugation as follows (*2*). Two 12-ml sucrose layers (1.2 and 0.8*M*) were overlain with 12 ml of whole-brain homogenate in 0.32*M* sucrose and centrifuged at 100,000*g* for 90 minutes. Three-milliliter fractions were collected and assayed for enzyme activity (Fig. 1). Peaks of enzyme activity were seen in the supernatant and "synaptosomal" areas.

The substrates included 5-hydroxytryptamine, tryptamine, *N*-methyltryptamine, 5-methoxytryptamine, 5-hydroxy-*N*,*N*-methyltryptamine, and norepinephrine. A portion (10 μg) of the enzyme purified on the Sephadex column was incubated with 5 μmole of the various substrates in 0.5 ml of incubation mixture, the optimum concentration of substrate, as shown with 5HT. Relative enzyme specificity was highest for this substance. The relative activity of this methyl transferase for various substrates is represented in Table 1. Under these reaction conditions, this enzyme is specific for methylating the amine nitrogen of indole(ethyl)amine substrate rather than the oxygen in the 5-position. Radioactive bufotenine, *N*-methyltryptamine and dimethyltryptamine chromatographed (thin layer) with known compounds on cellulose in isopropanol, ammonia, water (200:10:20); in 20 percent KCl in water; and in methanol, butanol, benzene, water (40:30:20:10).

We earlier attempted to explain the observed reversal of sedation by indoleamino acid after preliminary treatment with a monoamine oxidase inhibitor as being a result of the increased conversion of ^{14}C-5-hydroxytryptophan to bufotenine in the chick (*3*). The presence in brain of an indole(ethyl)amine *N*-methyltransferase that can convert indoleamines to centrally active *N*-methyl derivatives may account for this reversal. If such an enzyme is present in human brain, it might help explain Glassman's (*4*) evidence demonstrating the potentiation of the antidepressant effects of monoamine oxidase inhibitors with tryptophan loads. The presence of such an enzyme might also explain the mechanism of action of the monoamine oxidase inhibitors. In addition, this may account for the occasional psychotic reactions seen clinically after patients are treated with a monoamine oxidase inhibitor, or with amphetamines in doses high enough to result in monoamine oxidase inhibition (*5*).

We have recently demonstrated the presence of this enzyme in the brain of rat, with the highest specific activity in the brain stem and the lowest in cortical areas. In addition, we have evidence of the presence of this enzyme in infant parietal and adult frontal cortical tissue taken incidentally from man during neurosurgical procedures.

MERRILY MORGAN
ARNOLD J. MANDELL
Department of Psychiatry, University of California, San Diego, La Jolla 92037

References and Notes

1. J. Axelrod, in *Transmethylation and Methionine Biosynthesis*, S. K. Shapior and F. Schlenk, Eds. (Univ. of Chicago Press, Chicago, 1965).
2. S. H. Snyder, J. Glowinski, J. Axelrod, *Life Sci.* **4**, 797 (1965).
3. C. E. Spooner, A. J. Mandell, D. Brunet, M. Cruickshank, I. M. Sabbot, *Fed. Proc.* **27**, 540 (1968); A. J. Mandell and C. E. Spooner, in *Schizophrenia, An Appraisal*, D. V. S. Sankar, Ed. (Pergamon, New York, 1969).
4. A. Glassman, paper read at annual meeting of the Psychiatric Research Society, Mental Health Center, New Haven, Conn., 5 December 1968.
5. L. L. Iversen, J. Axelrod, J. Glowinski, in *Neuropsychopharmacology*, H. Brill, J. O. Cole, P. Deniker, H. Hippius, P. B. Bradley, Eds. (Excerpta Medica Foundation, New York, 1967).
6. Supported by NIMH grant MH-14360-02. We thank J. Biel of Aldrich Chemical Co. for supplying substrates and carrier indoles.

28 April 1969

2-p-Toluidinyl-6-Naphthalene Sulfonate: Relation of Structure to Fluorescence Properties in Different Media

Abstract. *Hydrated and anhydrous crystals of 2-p-toluidinyl-6-naphthalene sulfonate exhibit fluorescence spectra similar to those obtained for 2-p-toluidinyl-6-naphthalene sulfonate in water and in organic solvents, respectively. The molecular structure of anhydrous 2-p-toluidinyl-6-naphthalene sulfonate suggests extended electron resonance over the whole molecule and this is one of the causes of the spectral differences.*

The fluorescence properties of 2-*p*-toluidinyl-6-naphthalene sulfonate (TNS) are dependent upon the polarity and steric structure of the environment (*1*). It is practically nonfluorescent in water but fluoresces with different quantum yields when dissolved in organic solvents and when noncovalently bound to different native proteins (*2*). This property makes TNS useful as a probe for determining polarity of protein active sites and as a fluorescent monitor of substrate binding and enzyme activation processes which entail protein conformational changes (*3*, *4*). The variation of fluorescence characteristics with environment is not well understood and we have undertaken structural studies of TNS to provide more data on this problem.

Crystallization was effected by slow diffusion of water into a solution of TNS potassium salt dissolved in *N*,*N*-dimethylformamide. Two types of crystals (A and B) were obtained. Type A contains water in the crystal lattice, and upon removal from solution type A loses the water and changes to lattice type B; in the process the crystals crack

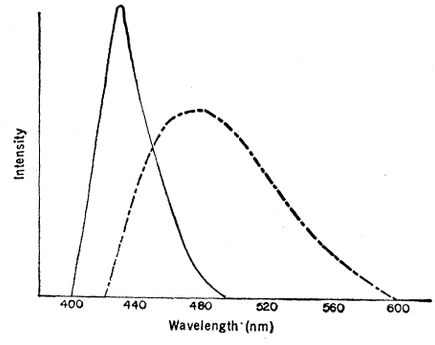

Fig. 1. Fluorescence spectra of TNS crystals. Solid line indicates anhydrous crystals; broken line indicates hydrated crystals.