Biochemical Pharmacology, Vol. 25, pp. 2251–2256. Pergamon Press, 1976. Printed in Great Britain.

INHIBITION OF INDOLEAMINE-*N*-METHYLTRANSFERASE BY 2,3,4,6,7,8-HEXAHYDROPYRROLO[1,2-a]PYRIMIDINE

LEWIS R. MANDEL

Merck Institute for Therapeutic Research, Rahway, N.J. 07065, U.S.A.

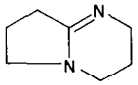
(*Received* 13 *November* 1975; *accepted* 5 *March* 1976)

Abstract—2,3,4,6,7,8-Hexahydropyrrolo[1,2-a]pyrimidine is a potent inhibitor *in vitro* of indoleamine-*N*-methyltransferase, an enzyme present in animal and human lung catalyzing the conversion of *N*-methyltryptamine to *N*,*N*-dimethyltryptamine. It is a reversible inhibitor, non-competitive with respect to *N*-methyltryptamine. The compound does not block the activity of phenethanolamine- or imidazole-*N*-methyltransferases *in vitro*. When administered orally to rabbits, 2,3,4,6,7,8-hexahydropyrrolo[1,2-a]pyrimidine markedly reduces the specific activity of the lung indoleamine-*N*-methyltransferase. This inhibition of enzyme activity is accompanied by a block in the conversion of intravenously administered ^{14}C-labeled *N*-methyltryptamine to [^{14}C]dimethyltryptamine in lung and brain.

The *N*,*N*-dimethylindoleamines are hallucinogenic agents and one of these compounds, *N*,*N*-dimethyltryptamine (DMT), is a potent psychotogen in man [1–3]. DMT is the most well-studied human hallucinogen for which there is a biosynthetic mechanism. Axelrod [4] first reported on the occurrence of the DMT-forming enzyme in rabbit lung, and indoleamine-*N*-methyltransferase (INMT) has since been reported to be present in several tissues from laboratory animals and man [5–11].

A variety of naturally occurring and synthetic inhibitors of INMT have also been described. Inhibitors have been found in rat brain [6], human lung [10], human blood [9], rabbit lung [12] and in bovine pineal extracts [13]. Preliminary studies indicate that these inhibitors are low molecular weight materials [13]. The first synthetic compounds reported to inhibit lung INMT *in vitro* were chlorpromazine and imipramine [4]. More recently, nor$_1$ and nor$_2$ chlorpromazine [14], *S*-adenosylhomocysteine [15] and 3,4-dihydroxyphenylacetic acid [16] have been described as inhibitors of INMT activity. Relatively high levels of the substrates *N*-methyltryptamine (NMT) and 5-methoxy-NMT, and of the reaction products DMT and bufotenin (*N*,*N*-dimethylserotonin) have also been found to inhibit enzyme activity *in vitro* [17, 18].

This paper describes a new bicyclic INMT inhibitor, 2,3,4,6,7,8-hexahydropyrrolo[1,2-a]pyrimidine (Fig. 1). 1,5-Diazabicyclo[4.3.0]non-5-ene (DBN) is the name employed by the Aldrich Chemical Co., from whom this compound was obtained. DBN was discovered as a result of screening efforts in this laboratory. *In vitro*, this compound is a potent inhibitor of INMT from rabbit, monkey and human lung; it does not inhibit other *N*-methyltransferases. *In vivo*, it is orally active in blocking the conversion of ^{14}C-labeled NMT to [^{14}C]DMT in rabbit lung and brain.



2,3,4,6,7,8-Hexahydropyrrolo [1,2-a] pyrimidine
(1,5-diazabicyclo [4.3.0] non-5-ene; DBN)

Fig. 1. Structure and nomenclature of DBN.

MATERIALS AND METHODS

INMT was partially purified from rabbit and human lung according to methods described previously [10, 17]. Crude preparations of the enzyme were prepared from other tissues (rabbit brain, rat and human liver, and monkey lung) by centrifuging the tissue homogenates at 78,000 *g* for 1 hr at 0° and dialyzing the supernatants against 50–200 vol. of 10^{-4} M sodium phosphate buffer, pH 7·9, for 20 hr at 4°. In some cases EDTA and dithiothreitol (5×10^{-5} M) were added to the buffer. When a precipitate formed upon dialysis it was removed by centrifugation at 25,000 *g* for 15 min at 0°. Assays of INMT were carried out as outlined earlier [10, 17, 18]. Bovine adrenal phenethanolamine-*N*-methyltransferase was partially purified and assayed by the method of Axelrod [19] using normetanephrine as substrate. Imidazole-*N*-methyltransferase activity in rabbit lung was assayed according to the procedure of Brown *et al.* [20] using histamine as substrate. Protein was measured by the method of Oyama and Eagle [21].

For the experiments *in vivo*, rabbits were given DBN (as the free base or as the fumarate salt, pH adjusted to 6) by i.v. injection, by maintaining the animals on the compound in the drinking water or by intubating single oral doses with a No. 8 or No. 10 French catheter. Crude INMT was then prepared as follows. The rabbits were decapitated, bled out and the lungs were placed in 50 ml of 0.15 M KCl at 0° for 15 min. The tissue was then homogenized in 5–10 vol. of fresh KCl solution in a Waring Blender for 1 min. The homogenates were centrifuged at 78,000 *g* for 20–45 min at 0° and the supernatants assayed directly for INMT activity using NMT as the indoleamine substrate.

In the experiments designed to measure the conversion *in vivo* of ^{14}C-labeled NMT to [^{14}C]DMT, control and DBN-treated rabbits were given the monoamine oxidase inhibitor pheniprazine (10 mg/kg, i.v.) After 6 hr they were dosed i.v. (ear vein) with ^{14}C-labeled NMT. Five min after the injection of NMT, the animals were decapitated and the lungs and brains frozen in liquid nitrogen at once. [^{14}C]DMT was subsequently isolated from the tissues by minor modification of the solvent extraction procedure previously described [22]. The [^{14}C]DMT was determined by thin-layer chromatography of the final isolate on Silica gel GF plates using a developing solvent of methanol–1 N NH_4OH (5:1). The DMT was recovered from the Silica gel plates by elution with methanol and the radioactivity determined in a Packard liquid scintillation counter. In some experiments the identification of the [^{14}C]DMT was confirmed by reverse isotope dilution analysis [23].

S-adenosylmethionine-methyl [^{14}C] (sp. act. 42–53 mCi/m-mole) was purchased from the New England Nuclear Corp. All the indoleamines (as their free bases and/or salts) and DBN were purchased from the Aldrich Chemical Co. Normetanephrine HCl and histamine diHCl were obtained from CalBiochem. Pheniprazine (Catron) was kindly donated by Lakeside Laboratories. 5-Methoxy-NMT was synthesized by the method of Wilkinson [24] and ^{14}C-labeled NMT by the method of Horner and Skinner [25]. All other chemicals were reagent grade commercial products. The rabbits used in these studies were New Zealand white (NZW) males purchased from H.A.R.E., West Milford, N.J.

RESULTS

In the initial experiments, the inhibitory effect of DBN on the activity of rabbit lung INMT, purified through the Sephadex G-150 step, was evaluated. Reaction mixtures contained 31 μmoles potassium phosphate buffer, pH 7.9; 2.4 nmoles *S*-adenosylmethionine-methyl[^{14}C] (160,000 cpm); and 34 nmoles NMT; enzyme and DBN in a final volume of 0.1 ml. Mixtures were incubated for 60 min at 37°, and the reaction was terminated with 0.2 ml of 0.125 M sodium tetraborate, pH 10. [^{14}C]DMT was extracted into 2 ml of water-saturated isoamyl alcohol and the radioactivity in 0.5 to 1.0-ml aliquots was determined. As shown in Fig. 2a, DBN produced nearly complete inhibition of enzyme activity at a concentration of 2×10^{-4} M and a small but significant inhibition at 4×10^{-7} M. Inhibition of the purified enzyme by DBN is apparently non-competitive with respect to NMT; the K_i value is of the order of 2×10^{-6} M (Fig. 2b). At 2×10^{-5} M, DBN was found to inhibit the methylation of serotonin and *N*-methylserotonin 80 per cent, and at 2×10^{-4} M it blocked the methylation of 5-methoxytryptamine and 5-methoxy-NMT to their corresponding *N*-methylated products about 90 per cent. Thus, DBN *in vitro* is an inhibitor of the biosynthesis of three indoleamines reported to affect behavior in laboratory animals or man: DMT [3], 5-methoxy-DMT [26] and *N,N*-dimethylserotonin [27]. The effect of DBN on the methylation of other amines was not studied.

DBN was also evaluated for its inhibitory effect on DMT-forming enzymes from sources other than rabbit lung. In these experiments the co-substrates were 2–5 mM NMT and 8–24 μM *S*-adenosylmethionine-methyl[^{14}C]. The incubation period was 1–2 hr at 37°. The reactions were terminated by the addition of borate, pH 10, and the [^{14}C]DMT was extracted into either water-saturated isoamyl alcohol or toluene–isoamyl alcohol (97:3). In most of the experiments, the [^{14}C]DMT was isolated by thin-layer chromatography on Silica gel GF plates with a developing solvent of either *n*-butanol–acetic acid–water (72:18:30; R_f DMT = 0.53) or methanol–1 N NH_4OH (5:1; R_f DMT = 0.41). As shown in Table 1, DBN markedly inhibited the activity of enzyme preparations from rabbit liver and brain, rhesus monkey and human lung, but not rat or human liver. The insensitivity of the enzymes from rat and human liver to inhibition by DBN suggests these two enzymes may be different from all others studied. None of the enzymes listed in Table 1 were inhibited by the phenethanolamine-*N*-methyltransferase inhibitor 5,6-dichloro-2-aminobenzimidazole [28] at 10^{-4} M indicating that they are not similar to the adrenal methyltransferase. No enzyme activity was found in rat or human brain extracts, in contrast to the findings of others [5, 6], and consequently DBN could not be evaluated on enzymes from these sources. DBN was equally effective in blocking the *N*-methyla-

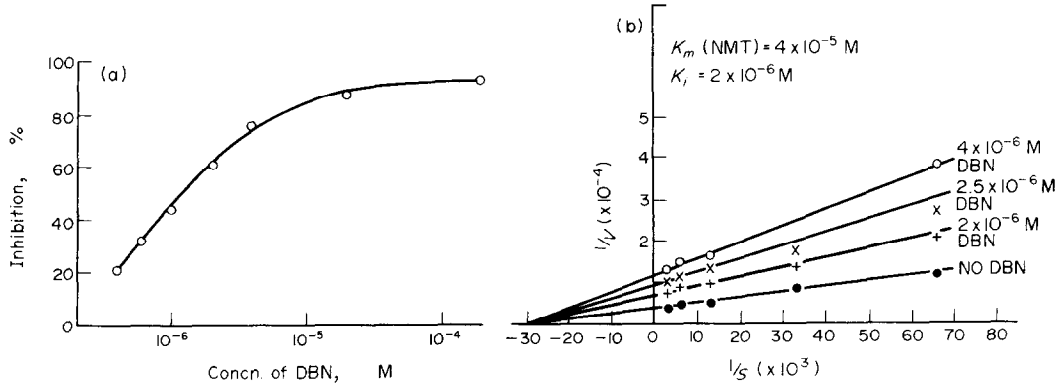


Fig. 2. (a) Dose response curve *in vitro* for inhibition of partially purified rabbit lung INMT by DBN. (b) Kinetic analysis of INMT inhibition *in vitro* produced by DBN.

Table 1. Inhibition of DMT-forming enzymes by DBN

Enzyme preparation*	Per cent inhibition of [^{14}C]DMT formation by DBN†		
	2×10^{-4} M	2×10^{-5} M	2×10^{-6} M
1. Rhesus monkey lung	85		
2. Human lung	71	44	9
3. Human lung (purified through Sephadex G-150 step)	91	68	37
4. Rabbit liver	100		
5. Rat liver	20		
6. Human liver	21		
7. Rabbit brain		83	65

* With the exception of enzyme No. 3, all preparations were dialyzed 78,000 × *g* supernatants of tissue homogenates.

† Data are for DBN as the free base except for enzyme No. 3, where DBN fumarate was used.

tion of serotonin by human lung INMT as it was in blocking the methylation of NMT (data not shown).

To determine whether the inhibitory effect of DBN was specific for INMT-type activity and that it was not a non-specific inhibitor of other *N*-methyltransferases, it was tested for its effect on bovine adrenal phenethanolamine-*N*-methyltransferase, and rabbit and human lung imidazole-*N*-methyltransferase, enzymes which also require *S*-adenosylmethionine as the methyl donor and which have no metal ion requirement. As shown in Table 2, DBN at levels up to 100 times its K_i against rabbit lung INMT produced no significant inhibition of the *N*-methylation of normetanephrine or histamine. Its effect on *O*-methyltransferases was not evaluated. Because DBN is a strong organic base and could possibly interact with *S*-adenosylmethionine in the assays *in vitro*, 2–20 nmoles was incubated with 2 nmoles of radioactive methyl donor (1.7×10^5 cpm) in the absence of enzyme for 1 hr at 37° (pH 7.5). All the radioactive *S*-adenosylmethionine was recovered after thin-layer chromatography in butanol–acetic acid–water (60:15:25), indicating that DBN does not block INMT activity indirectly by reducing the availability of methyl donor.

Experiments designed to evaluate whether DBN inhibits INMT activity *in vivo* were then carried out. NZW male rabbits (1.5 to 1.8 kg) were given single doses of the compound (0.3 to 30 mg/kg) in the ear vein. At various times after dosage the animals were decapitated and bled out, and a crude INMT preparation was made from the lung homogenates and assayed *in vitro* as outlined earlier. The data in Table 3 demonstrate that the specific activity of the INMT is reduced to about 25 per cent of control values after single i.v. doses of 3–30 mg/kg of DBN when the rabbits are sacrificed 10 min post injection. Enzyme activity is depressed 1–4 hr after dosage but the data are variable at the 4-hr time point. In one rabbit, DBN at 0.3 mg/kg was only marginally effective in lowering the activity of INMT.

To establish whether DBN was orally active in reducing INMT activity, rabbits were given 0.5% DBN (as the free base or the fumarate salt at pH 6) in the drinking water for several days. The animals (three/group) consumed 50–150 ml of medicated water (250–750 mg DBN)/day. This treatment for 6–8 days resulted in about a 90 per cent depression of enzyme activity (the average specific activity of INMT for the controls was 76,000 cpm DMT/mg of protein/hr, while the average value for the DBN-treated rabbits was 7,900). Even 3–5 days of maintenance on DBN resulted in a marked (60–80 per cent) decrease in the activity of the DMT-forming enzyme. To confirm the oral activity of DBN, a second series of experiments was carried out in which rabbits (fasted for 16–20 hr) were given single oral doses of the compound with a stomach tube. Animals were sacrificed at various times after dosage and measurements of

Table 2. Effect of DBN on *N*-methyltransferases *in vitro**

Concn of DBN	Activity (cpm)				
	INMT		Phenethanolamine-*N*-methyltransferase	Imidazole-*N*-methyltransferase	
	Rabbit lung	Human lung	Bovine adrenal	Rabbit lung	Human lung
None	16,300	2,580	18,100	22,500	10,000
2×10^{-4} M	2,291 (91)	530 (79)	17,300 (5)	21,700 (4)	9,900 (0)
2×10^{-5} M	3,179 (86)	1,170 (53)	17,700 (3)	26,076 (+15)	
2×10^{-6} M	6,633 (64)		18,380 (0)	21,338 (5)	

* Data are expressed as cpm in the product and the figures in parentheses are the per cent inhibition values. NMT was the substrate for INMT, normetanephrine HCl was used for phenethanolamine-*N*-methyltransferase and histamine dihydrochloride for the imidazole-*N*-methyltransferase.

Table 3. Effect of DBN (i.v.) on the activity of rabbit lung INMT

Dosage of DBN (mg/kg)	No. of rabbits/group	Time of sacrifice after dosage	Sp. act. of INMT*
Control	3		72,000; 77,000; 88,000
30	2	10 min	15,000; 16,200 (79)†
30	3	1 hr	23,000; 26,000; 26,000 (67)
30	3	4 hr	36,000; 32,000; 55,000 (46)
3	2	10 min	19,000; 21,000 (73)
3	2	1 hr	46,000; 48,000 (39)
0.3	1	10 min	67,000 (11)

* Data are expressed as cpm DMT formed *in vitro*/mg of enzyme protein/hr at 37°.
† Figures in parentheses are the average per cent inhibition values.

lung enzyme activity were made by the standard procedure. As shown in Table 4, single doses of 300 mg/kg of DBN resulted in an 84 per cent reduction in the specific activity of INMT. At 60 mg/kg there was over a 60 per cent depression of activity but the results were less consistent, particularly at the 4-hr time point. Lower doses of DBN (25 mg/kg) produced no inhibition of enzyme activity 2 hr after oral dosage.

Studies designed to determine whether the inhibition of the lung INMT by DBN was reversible were also performed. Enzyme preparations from a rabbit given DBN fumarate (300 mg/kg orally) and a control rabbit were dialyzed against 3000 vol. of 1 mM potassium phosphate buffer, pH 7, for 20 hr at 4°. Enzyme activity was assayed before and after dialysis, and as shown in Fig. 3, dialysis had little effect on the activity of the control enzyme but the specific activity of the INMT from the DBN-treated rabbit returned to near control values. These results suggest that DBN produces a reversible type of inhibition, i.e. the drug–enzyme complex is dissociable. A similar pattern of results was obtained when DBN (2×10^{-5} M) was added to a control enzyme *in vitro* and the preparation dialyzed. Re-addition of DBN (2×10^{-5} to 2×10^{-6} M) to dialyzed enzymes produced the expected inhibition (45–70 per cent) of enzyme activity.

Experiments were devised to determine whether inhibition of INMT activity in DBN-treated rabbits was accompanied by an inhibition in the biosynthesis *in vivo* of DMT. Rabbits (400–800 g) were given 0.5% DBN in the drinking water for 5–6 days; under these conditions INMT activity was depressed about 75 per cent. The animals were treated with the monoamine oxidase inhibitor pheniprazine (10 mg/kg, i.v.) and 6 hr later they were given 25–28 μCi of ^{14}C-labeled NMT i.v. Five min after injection of label the rabbits were decapitated and the lungs and brains removed and frozen at once in liquid nitrogen (−196°). [^{14}C]-DMT was subsequently extracted quantitatively from the tissues by minor modification of the solvent extraction procedure used to isolate DMT from human blood [22]. Carrier DMT (100 μg) was added at the outset of the isolation procedure and the [^{14}C]-DMT was determined by thin-layer chromatography as outlined in Methods. The data in Table 5 demonstrate that under conditions where INMT is reduced, there is a concomitant block in the conversion *in vivo* of NMT to DMT. In lung, this block was 80–96 per cent while in brain it was 33 and 87 per cent. In Expt. No. 1, the [^{14}C]NMT in lung and brain was also isolated; the average value was increased in the DBN-treated rabbits by 49 and 66 per cent, respectively, over controls. This accumulation of NMT is consistent with an inhibition of INMT activity. Because the content of [^{14}C]DMT in brain was low, another experiment was performed in which control rabbits were given the [^{14}C]NMT (13 μCi; 0.3 mg) by intracisternal injection. One animal was sacrificed 5 min after injection, another at 10 min and the third at 20 min. However, the DMT values remained low at 177, 95 and 60 cpm/g of brain respectively. In other experiments with 3-kg rabbits given DBN fumarate (0.6% in the drinking water for 5–6 days), there was about a 90 per cent inhibition in the conversion of

Table 4. Specific activity of rabbit lung INMT after single oral doses of DBN

Dosage of DBN (mg/kg)	No. of rabbits/ group	Time of sacrifice after dosage (hr)	Sp. act. of INMT*
Control†	3	2	64,000; 66,000; 76,000
300	1	1	11,000 (84)‡
300	1	2	10,000 (85)
300	1	4	11,000 (84)
60	3	1	20,000; 22,000; 28,000 (66)
60	2	2	14,000; 28,000 (69)
60	2	4	18,000; 30,000; 32,000 (61)
25	2	2	95,000; 86,000

* Data are expressed as cpm DMT formed *in vitro*/mg of enzyme protein/hr at 37°.
† These animals were given water with the stomach tube.
‡ Figures in parentheses are the average per cent inhibition values.

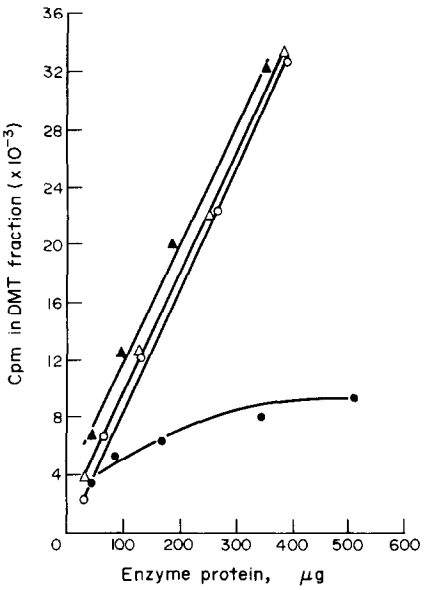


Fig. 3. Inhibition *in vivo* of rabbit lung INMT activity and reversibility of inhibition by DBN. Key: (▲——▲) control enzyme not dialyzed; (○——○) control enzyme dialyzed; (△——△) enzyme from DBN-treated rabbit, dialyzed; and (●——●) enzyme from DBN-treated rabbit, not dialyzed.

intravenously administered NMT (7 mg/kg) to DMT, measured in carotid arterial blood by a minor modification of the method of Walker *et al.* [22]. Control animals produced 22–35 ng DMT/ml of blood over the first minute after injection of NMT while rabbits that consumed 15 g of drug over the pretreatment period produced only 1 3 ng DMT/ml. In these studies, two rabbits/group were used; each animal was given NMT three times at 20 to 30-min intervals.

DISCUSSION

The studies described in this paper demonstrate that DBN is a potent inhibitor of the methyltransferase which catalyzes the formation of DMT, a psychotomimetic agent [1]. It also inhibits the biosynthesis *in vitro* of 5-methoxy-DMT and *N,N*-dimethylserotonin (bufotenin), two other structurally related compounds implicated to play a role in mental disease [26, 27]. Inhibition of INMT activity by DBN has also been demonstrated to occur *in vivo* and this was accompanied by a block in the biosynthesis of DMT from its precursor NMT.

DBN has no effect on the activity of phenethanolamine- or imidazole-*N*-methyltransferases, or on the activity of a DMT-forming enzyme present in human and rat liver. Moreover it has no activity in other assay systems. As examples, DBN does not affect adenylcyclase activity, indole acid acetic biosynthesis, or 5-methyltetrahydrofolate-catalyzed pyrido-indole formation *in vitro* (L. R. Mandel and M. Malkin, unpublished results). DBN *in vivo* has no antidepressant activity in laboratory animals and no effect on a Sidman avoidance schedule in the squirrel monkey, and produces no noteworthy behavioral signs in rats, mice or monkeys (H. Hanson, unpublished observations). Analogs of DBN have been reported in the patent literature to have anti-inflammatory, anti-convulsive, analgesic and hypotensive effects [29]. With the exception of our earlier report [30], there are no other patents or publications citing biological activities for DBN *per se*.

Because DBN blocks the biosynthesis of DMT it would be of interest in the treatment of schizophrenia. There is suggestive evidence that DMT may be involved in some phases of this disease. In man, intramuscular administration of DMT produces some of the symptoms of acute schizophrenia [3]. Administration to schizophrenics of amino acid precursors of DMT with monoamine oxidase inhibitors appears to exacerbate symptomatology [31]. There is an enzymatic mechanism for the formation of DMT in man [5–10]. At the present time, there is no animal model available to evaluate a DMT biosynthesis inhibitor as a potential new treatment for schizophrenia. To test the DMT hypothesis, an inhibitor with the mode of action of DBN would have to be evaluated in

Table 5. Effect of DBN on the *in vivo* conversion of [^{14}C]NMT to [^{14}C]DMT in rabbit lung and brain*

Expt. No.	Animal No.	Activity (cpm in [^{14}C]NMT/g tissue)		Activity (cpm in [^{14}C]DMT/g tissue)	
		Lung	Brain	Lung	Brain
1	Control 1	257,000	6,800	14,200	103
	Control 2	200,000	9,200	10,800	122
	Control 3	180,000	6,050	7,450†	127
	DBN 1	252,000	10,200	1,190	74
	DBN 2	400,000	11,400	3,300	78
	DBN 3	295,000 (49)‡	15,000 (66)‡	2,250 (80%)§	87 (33%)§
2	Control 1			5,591	56
	Control 2			4,659	68
	Control 3			5,776	84
	DBN 1			153	6
	DBN 2			219 (96%)§	11 (87%)§

* In Expt. 1, 600 to 800-g rabbits were maintained on 1% DBN fumarate (equivalent to 0.5% DBN free base) for 6 days. [^{14}C]NMT [28 μCi (0.7 mg)] was given to the rabbits. In Expt. 2, animals (400–500 g) were given 0.5% DBN for 5 days and 25 μCi (0.5 mg) of [^{14}C]NMT was administered.

† Reverse isotope dilution analysis demonstrated that this sample was ≥85% DMT.

‡ Figures in parentheses are the average percentage increase values.

§ Figures in parentheses are the average per cent inhibition values.

schizophrenic patients. Whether a novel therapeutic agent will be the outcome of such a study remains to be established.

Acknowledgements—The author is grateful to Dr. E. Plocharski, Dr. H. S. Ahn, Mr. R. Walker, Mr. B. Lopez-Ramos and Miss M. Galavage for valuable assistance on various aspects of this work. The author thanks Dr. Avery Rosegay for the synthesis of the ^{14}C-labeled *N*-methyltryptamine and the 5-methoxy-NMT.

REFERENCES

1. S. Szara, in *Amines and Schizophrenia* (Eds. H. E. Himwick, S. S. Kety and J. R. Smythies), p. 181. Pergamon Press, Oxford (1967).
2. A. T. Shulgin, *Neurosci. Res. Prog. Bull.* **8,** 72 (1970).
3. R. J. Wyatt, J. C. Gillin, J. Kaplan, R. Stillman, L. R. Mandel, H. S. Ahn, W. J. A. VandenHeuvel and R. W. Walker, *Adv. Biochem. Psychopharmac.* **11,** 299 (1974).
4. J. Axelrod, *J. Pharmac. exp. Ther.* **138,** 28 (1962).
5. A. J. Mandell and M. Morgan, *Nature New Biol.* **230,** 86 (1971).
6. J. M. Saavedra and J. Axelrod, *Science, N.Y.* **175,** 1365 (1972).
7. B. Heller, *Psychosomatics* **12,** 273 (1971).
8. N. Narasimhachari, J. M. Plaut and H. E. Himwich, *Life Sci.* **11,** 221 (1972).
9. R. J. Wyatt, J. M. Saavedra and J. Axelrod, *Am. J. Psychiat.* **130,** 754 (1973).
10. L. R. Mandel, H. S. Ahn, W. J. A. VandenHeuvel and R. W. Walker, *Biochem. Pharmac.* **21,** 1197 (1972).
11. E. Meller, H. Rosengarten and A. J. Friedhoff, *Life Sci.* **14,** 2167 (1974).
12. R.-L. Lin, S. Sargeant and N. Narasimhachari, *Dev. Psychobiol.* **7,** 745 (1974).
13. N. Narasimhachari, R.-L. Lin and H. E. Himwich, *Res. Commun. Chem. Path. Pharmac.* **9,** 375 (1974).
14. N. Narisimhachari and R.-L. Lin, *Res. Commun. Chem. Path. Pharmac.* **8,** 341 (1974).
15. R.-L. Lin, N. Narisimhachari and H. E. Himwich, *Biochem. biophys. Res. Commun.* **54,** 751 (1973).
16. P. C. Waldmeier and L. Maitre, *Experientia* **30,** 456 (1974).
17. L. R. Mandel, S. Rosenzweig and F. A. Kuehl, Jr., *Biochem. Pharmac.* **20,** 712 (1971).
18. L. R. Mandel and R. W. Walker, *Life Sci.* **15,** 1457 (1974).
19. J. Axelrod, *J. biol. Chem.* **237,** 1657 (1962).
20. D. D. Brown, R. Tomchick and J. Axelrod, *J. biol. Chem.* **234,** 2948 (1959).
21. I. Oyama and H. Eagle, *Proc. Soc. exp. Biol. Med.* **91,** 305 (1958).
22. R. W. Walker, H. S. Ahn, G. Albers-Schonberg, L. R. Mandel and W. J. A. VandenHeuvel, *Biochem. Med.* **8,** 105 (1973).
23. R. W. Walker, H. S. Ahn, L. R. Mandel and W. J. A. VandenHeuvel, *Analyt. Biochem.* **47,** 228 (1972).
24. S. Wilkinson, *J. chem. Soc.* 2079 (1958).
25. J. K. Horner and W. A. Skinner, *Can. J. Chem.* **44,** 315 (1966).
26. J. R. Smythies, R. J. Bradley and U. S. Johnston, *Life Sci.* **6,** 1887 (1967).
27. H. D. Fabing and J. R. Hawkins, *Science, N.Y.* **123,** 886 (1956).
28. L. R. Mandel, C. C. Porter, F. A. Kuehl, Jr., N. P. Jensen, S. M. Schmitt, T. B. Windholz, T. R. Beattie, J. A. Carty, B. G. Christensen and T. Y. Shen, *J. med. Chem.* **13,** 1043 (1970).
29. N. M. Bortnick, O. Fegley and M. F. Fegley, U.S. Patents No. 2,984,665 (May 16, 1961), 2,993,046 and 2,993,049 (July 18, 1961); Geigy, British Patent No. 1,038,735 (Aug. 10, 1966).
30. L. R. Mandel, U.S. Patent No. 3,749,781 (July 31, 1973).
31. S. S. Kety, *New Engl. J. Med.* **276,** 325 (1967).