marijuana smoking does not produce a gross cellular immune defect that can be detected by skin testing. Further study is needed to evaluate chronic marijuana use and its effect, if any, on the immune system.

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4. Marijuana smoking ranged from three times per week to several times per day. No marijuana was administered by us to these subjects, so the specific dosage levels or percentage of tetrahydrocannabinol of the marijuana smoked is not known.
5. Statistical analyses performed were the Yates modification of the chi-square procedure and Fisher's exact probability test (two-tailed).
6. $\chi^2 = 0.18$, $P = .669$; Fisher's exact $P = 1.000$.
7. $\chi^2 = 7.84$, $P = .005$; Fisher's exact $P = .001$.
8. $\chi^2 = 3.68$, $P = .055$; Fisher's exact $P = .030$.
9. $\chi^2 = 2.13$, $P = .144$; Fisher's exact $P = .133$.
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5-Methyltetrahydrofolic Acid as a Mediator in the Formation of Pyridoindoles

Abstract. *Enzymes from chick and rat tissues catalyze the reaction of* N-*methyltryptamine with 5-methyltetrahydrofolic acid to form 2,3,4,9-tetrahydro-2-methyl-1*H-*pyrido[3,4*b*]indole.* N,N-*Dimethyltryptamine was not formed. With tryptamine as substrate the product is 2,3,4,9-tetrahydro-1*H-*pyrido[3,4*b*]indole and not* N-*methyltryptamine. These pyridoindoles were not formed when* S-*adenosylmethionine was cosubstrate.*

5-Methyltetrahydrofolic acid (MTHF) transfers its methyl group to dopamine in a reaction catalyzed by an *N*-methylating enzyme to form epinine (*1*). More recently it was reported that MTHF is a more efficient cosubstrate than *S*-adenosylmethionine in the *N*- and *O*-methylation of certain amines (*2, 3*). We observed in our studies with MTHF that the product of methylation of tryptamine by MTHF was not isographic with *N*-methyltryptamine (NMT) in some of the solvent systems used for thin-layer chromatography (TLC), and that the product of the methylation of NMT by MTHF was not *N,N*-dimethyltryptamine (DMT). We now report evidence demonstrating that MTHF reacts with tryptamine and NMT to form tricyclic compounds.

Chick hearts from 2-week-old animals were homogenized in three to five volumes of 5 m*M* sodium phosphate buffer, *p*H 7.9 (with or without 0.01 m*M* dithiothreitol); the homogenate was centrifuged at 78,000*g* for 60 minutes. The supernatant fractions were dialyzed for 18 hours at 4°C against 50 volumes of buffer, with one change. Whole brains from rats (150 to 300 g) were homogenized in distilled water, and the 78,000*g* supernatant fraction was dialyzed against 20 m*M* potassium phosphate buffer, *p*H 6.8. The dialyzed preparations (4 to 8 mg of protein per milliliter) were the enzyme sources. Incubation mixtures (0.5 ml) contained 0.03 to 0.04 ml of enzyme, 3 to 10 m*M* indoleamine substrate (Aldrich), and 0.005 to 0.05 μmole of [^{14}C]MTHF ([5-methyl-^{14}C]tetrahydrofolate) (barium salt) (specific activity, 60 mc/mmole; Amersham/Searle) or 0.004 to 0.01 μmole of *S*-adenosyl[methyl-^{14}C]methionine (specific activity, 57 mc/mmole; New England Nuclear). Mixtures were incubated for 60 to 120 minutes at 37°C in the dark, and the reactions were terminated by addition of 0.6 ml of 0.5*M* borate at *p*H 10. The samples were extracted with 6 ml of 97 percent toluene and 3 percent isoamyl alcohol containing 50 to 100 μg of nonradioactive reference standard products. After shaking and centrifugation, a 5-ml portion of the organic phase was transferred to a vial, and the solvent was evaporated to dryness at 22°C in a vacuum desiccator. The residue was taken up in methanol for TLC on silica gel GF plates (Analtech). After development, the plates were examined under ultraviolet light, and scanned with a radiochromatogram scanner. The ultraviolet absorbing and radioactive zones were scraped into counting vials for determination of radioactivity in a liquid scintillation spectrometer.

NMT was labeled (in the benzene ring) with deuterium by exchange with CH_3SO_3D (60°C for 1 hour). 2,3,4,9-Tetrahydro-2-methyl-1*H*-pyrido[3,4*b*]indole (**1**) was prepared from 4,9-dihydro-

Table 1. Formation in vitro of compounds **1** and **2** from NMT or tryptamine and MTHF or *S*-adenosylmethionine (AMe).

Enzyme source	Indoleamine substrate	^{14}C-substrate		Radioactivity (count/min) in product zone*			
		Compound	^{14}C (10^5 count/min)	DMT	**1**	NMT	**2**
Chick heart	5 m*M* NMT	MTHF	4	371†			
				2	826		
Chick heart	5 m*M* NMT	MTHF	6.5	15	2010		
		AMe	7.5	40	22		
Chick heart	5 m*M* NMT	MTHF	15	55‡	6030‡		
		AMe	14	21‡	50‡		
Rat brain	5 m*M* NMT	MTHF	9	490†			
				14			
Rat brain	10 m*M* NMT	MTHF	10		2160‡		
Chick heart	5 m*M* Tryptamine	MTHF	7			10	1110
Rat brain	10 m*M* Tryptamine	MTHF	20			70	10,600
		AMe	8			10	80
Rat brain	10 m*M* Tryptamine	MTHF	10			20	2440‡

* Unless otherwise stated the number of counts per minute are the differences in the data obtained between the complete system and incubations in which the indoleamine was omitted. When the substrate was omitted, 40 to 330 count/min were found in the product zone. † The TLC solvent system was *n*-butanol, acetic acid, water (72 : 18 : 30); in all other experiments the solvent system was methanol, 1*N* NH_4OH (5 : 1). DMT and compound **1** were isographic (R_F 0.53) in the acid solvent system. ‡ In these experiments the enzyme was heated at 90°C for 15 minutes to assess the nonenzymatic reaction; 130 to 560 count/min were found in **1** and 140 to 420 count/min in **2**. The number of counts per minute represent the difference between the complete system and incubations with heat-denatured enzyme.

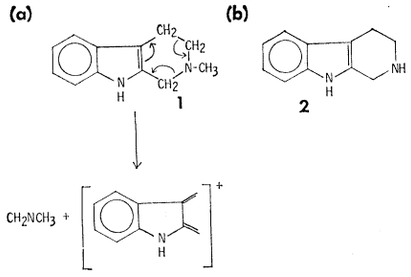

Fig. 1. (a) Compound **1**: 2,3,4,9-tetrahydro-2-methyl-1*H*-pyrido[3,4*b*]indole; (b) compound **2**: 2,3,4,9-tetrahydro-1*H*-pyrido[3,4,*b*]indole.

3*H*-pyrido[3,4*b*]indole (4) by sodium borohydride reduction of its methiodide, and 2,3,4,9-tetrahydro-1*H*-pyrido[3,4*b*] indole (**2**) by palladium carbon reduction of the same intermediate. [^{14}C]NMT was prepared by the procedure of Horner and Skinner (*5*); [^{14}C]DMT was synthesized from [^{14}C]indoleacetic acid by amidation and reduction.

As is shown in Table 1, the product (**1**) of the reaction of NMT with MTHF using enzymes from chick and rat tissues was isographic with DMT when the thin-layer chromatogram was developed in an acidic solvent system; however, in an alkaline system the radioactive zone showed an R_F of 0.7, different from that of DMT (0.41). *S*-Adenosylmethionine could not substitute for MTHF to form compound **1**. With the chick heart enzyme and ^{14}C-labeled NMT (3 m*M*; 2.4×10^6 count/min) and nonradioactive 0.3 m*M* MTHF (Sigma) as cosubstrates, the same compound (23,000 count/min) was also formed. To rule out the possibility that NMT and MTHF formed DMT which was then converted to another compound, ^{14}C-labeled DMT (60,000 count/min) was incubated under conditions similar to the standard assay; all the radioactivity was recovered as DMT. Moreover, DMT was not a substrate for the chick heart enzyme, indicating that methylation of the indole nitrogen by MTHF did not occur. When tryptamine was cosubstrate, the reaction product (**2**) was not isographic with NMT on TLC. The results in Table 1 indicate that the product formed from tryptamine is different from that proposed (*2*).

To establish the structure of compound **1** an experiment was performed in which nine reaction tubes each containing 0.35 ml of chick heart enzyme, 5 m*M* [D]NMT, ^{14}C-labeled MTHF (3×10^6 count/min), and 0.5 m*M* nonradioactive MTHF were incubated. [D]-NMT was used to provide a recognizable isotopic cluster in the mass spectrum of any substrate-derived product. The extracts were applied to TLC plates and developed in the methanol-NH_4OH system. Detection of the radioactive zone was achieved with a Packard radiochromatogram scanner and the product was recovered (50,000 count/min, total) by elution with methanol. Final purification was carried out by *p*H-dependent CH_2Cl_2 extractions.

Pertinent mass spectral data (LKB-9000 instrument) for NMT, [D]NMT, and the isolate are presented in Table 2. The ion of *m/e* 44 is $[CH_2NHCH_3]^+$, arising from the side chain. Because the deuterated substrate NMT is an isotopic mixture, [D]NMT and [D_2]NMT being the major components, characteristic isotope clusters are observed at the M and the M − 44/M − 43 regions. Comparable isotope clusters (therefore substrate related) are observed in the spectrum of the isolate at 12, not 14, mass units higher, ruling out a simple methylation of NMT. Thus, introduction of a methylene group with concomitant cyclization of the side chain is indicated; this proposal is supported by the absence of an ion of *m/e* 44 (and also *m/e* 58, the side chain fragment from DMT). A reasonable structure for compound **1** is 2,3,4,9-tetrahydro-2-methyl-1*H*-pyrido[3,4*b*]indole, and a mechanism for formation of the M − 43 ion is presented in Fig. 1a.

The mass spectrum of the synthesized compound **1** was compatible with that of the isolate. In addition to the mass spectral evidence, three other methods were used to establish the identity of the isolate with compound **1**. A sample consisting of the isolate (2000 count/min) and 5 μg of the synthetic compound was subjected to gas-liquid radiochromatography (1 percent W-98; 160°C; Barber-Colman model 5000). The mass and radioactivity peaks were coincident. Second, a sample consisting of the enzymatic isolate (1000 count/min) and 60 μg of the synthetic compound was subjected to TLC, with the use of the basic system. The ratios of radioactivity to mass for the applied sample at the leading and trailing portions of the zone of interest (R_F 0.72) were equivalent (16 count/min per microgram). Finally, reverse isotope dilution analysis (*6*) of the enzymatic isolate (3720 count/min) and the synthetic compound (25 mg) demonstrated that essentially all of the radioactive species (150 count/min per milligram) was compound **1**.

To demonstrate that the structure of compound **2** is 2,3,4,9-tetrahydro-1*H*-pyrido[3,4*b*]indole (Fig. 1b), extracts from incubations containing either chick heart or rat brain enzyme, 5 to 10 m*M* tryptamine, and ^{14}C-labeled MTHF (0.7×10^6 to 2.0×10^6 count/min) were applied to TLC plates and developed in the methanol-NH_4OH system. The radioactive zone (R_F 0.35; isographic with authentic **2**) was eluted with methanol, and 25 mg of compound **2** was added as carrier for reverse isotope dilution analysis; 95 percent of the radioactivity in the isolate was **2**.

Table 2. Pertinent mass spectral data.

Substance	M*	Fragment ions
NMT	174	M−44, M−43; 44
[D]NMT	175, 176	M−44, M−43; 44
Isolate	187, 188	M−43

* Molecular ion.

The mechanism for the formation of these pyridoindoles remains to be established. It seems improbable that there is a transfer of the methyl group from MTHF to NMT or tryptamine followed by oxidative cyclization since DMT is not a substrate. Reversal of the sequence producing MTHF to yield the precursor 5,10-methylenetetrahydrofolic acid and its subsequent reaction with the indoleamine is a possibility. Formation of the 5,10-methylene compound occurs in the presence of flavin adenine dinucleotide (*7*) and activation of the rat brain enzyme by the latter to form what is presumably **2** has been reported (*8*). Alternatively, the radioactive MTHF may be contaminated with traces of methylene sources. A nonenzymatic or artifactual component in the overall process of forming **1** and **2** could also be involved.

The pyridoindoles described herein and several related structures are known. Compound **1** has been isolated from plant sources (*9*). Both compounds have monoamine oxidase inhibitory activity (*10*). Although the functions of **1** and **2** are unknown, their precursors have been found in brain (*11*) and they may have a role in the nervous system. This is the first report on the biosynthesis in vitro of these pyridoindoles by enzymes of mammalian tissues.

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Reproductive Patterns in the Deep-Sea Benthos

Abstract. *The reproductive condition of a variety of benthic invertebrates from the bathyal San Diego Trough was monitored periodically. In this physically seasonless, deep-sea environment most species reproduce throughout the year, and only a few display highly synchronous, annual reproductive cycles. These few are not typical deep-sea species; they are known from shallow water and belong to groups which are relatively unimportant in abyssal depths. Of the 11 species examined, 3 bivalves, 2 ophiuroids, 2 isopods, 1 amphipod, and 1 polychaete breed year-round, and 1 brachiopod and 1 scaphopod spawn seasonally.*

The deep sea has long been considered the most constant of environments, the physical parameters changing only very slowly over thousands of years and displaying little or no seasonal variation (*1*). In shallow-water environments, most marine animals reproduce in a cyclic manner, seemingly in response to the demands of fluctuating external conditions (*2, 3*). Hence, it might be expected, as Orton (*4*) first suggested, that organisms inhabiting such a constant physical environment as the deep sea would reproduce throughout the year.

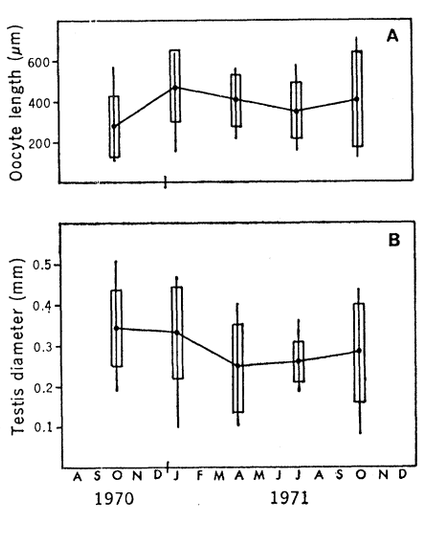
However, of the past attempts to investigate this question, indications of reproductive periodicity were found in a surprisingly large percentage of cases (*5*). Unfortunately, in all these instances the data are equivocal, having been derived from deep-sea bottom samples not specifically intended for reproduction studies. The samples were in most cases obtained from a variety of locations, depths, and years, often with samples either poor or lacking for several critical periods.

In order to avoid these difficulties in the investigation reported here, deep-sea populations were systematically monitored at regular intervals. Epibenthic sled (*6*) and otter trawl samples were taken every 13 weeks from October 1970 through October 1971, from the same station in the San Diego Trough (32°26.5′N, 117°28.5′W) at a depth of 1240 m.

The San Diego Trough is a relatively flat, sediment-filled basin 24 km off the coast of southern California. The sediments are a clayey-silt with a mean grain diameter of 8 μm (*7*) and an organic carbon content of 1 to 3 percent by weight (*8*). The three important physical factors—photoperiod, temperature, and salinity—changes in which are deemed to control the timing of reproduction in marine invertebrates (*2*), are nonexistent or do not vary throughout the year. Sunlight does not penetrate the depths of the trough (*9*) and the near-bottom temperature and salinity are nearly constant, ranging only 0.3°C and 0.02 per mil during the year (*10*).

Most of the species examined from this physically seasonless environment reproduce throughout the year. This is achieved in two different ways: (i) asynchronously, with the individuals of a population having distinct gametogenic cycles but being out of phase with each other, so that a relatively constant proportion of individuals breeds at any one time; and (ii) continuously, with all adult individuals in the population being reproductively active throughout the year. Only a few gametes are released at any one spawning so that complete spawn-out and recovery does not occur as it does in the asynchronous type.

Asynchronous year-round reproduction was seen among the echinoderms (*Ophiomusium lymani* and *Ophiacantha normani*) and the crustaceans (*Eurycope californiensis, Harpiniopsis excavata,* and *Ilyarachna profunda*). For example,



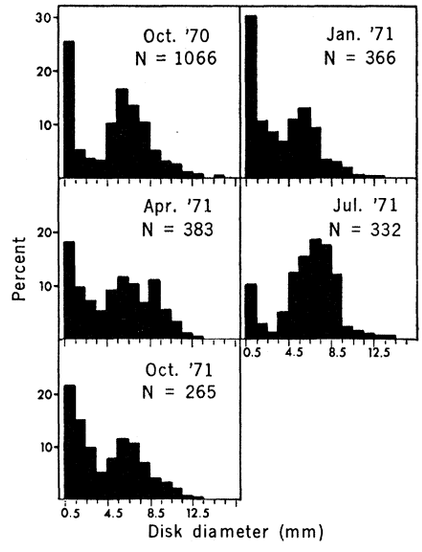


Fig. 1 (left). Year-round reproduction in the brittle star *Ophiacantha normani*. (A) Oocyte size of females during the year. (B) Testis size of males during the year. Each data point represents the mean for ten individuals. Oocyte length for an individual female was obtained by measuring the largest oocyte present in the gonads of one randomly selected genital bursa. Testis diameter for an individual male represents the mean of ten testes from one randomly selected genital bursa. A vertical line represents the 95 percent confidence limits of the mean. Fig. 2 (right). Size-frequency distributions of the sample population of *Ophiacantha normani*. Histograms for each season were prepared from epibenthic sled (*6*) samples.