limit of sensitivity of the bioassay, as applied in this study, was estimated to be 0.6 ng of melatonin per 8-hour urine sample.

In several, nonconsecutive 8-hour urine specimens collected over a month from the same six subjects, the mean amount of melatonin in the nighttime samples for each subject significantly exceeded the mean amounts observed in daytime samples (Fig. 1).

These observations confirm that at least a portion of the melatonin secreted from the human pineal finds its way chemically unchanged into the urine (*4*). They also demonstrate that (i) the rate of melatonin excretion, and possibly secretion, is greatest during nighttime (that is, at the time of day when melatonin synthesis is greatest in rats (*3*) and (ii) the total amount of melatonin excreted in a 24-hour period varies considerably among individuals. Further studies will be needed to identify the sources of this variation (such as age- or sex-related differences in the secretion of melatonin, or in its rate of metabolism and clearance) and to determine the extent to which the human melatonin rhythm is entrained by light-dark cycles, sleep, rhythms in motor activity, or food consumption (*14*).

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8. Salt saturation prevented the partition of isopropanol into the aqueous phase and allowed the alkaline and acid washes to be accomplished with minimal loss of melatonin.
9. The evaporation under nitrogen of multiple small samples of organic solvent was facilitated by the use of a plastic manifold equipped with interchangeable glass capillary tubes. The construction of such an assembly will be described elsewhere (H. J. Lynch, R. J. Wurtman, M. A. Moskowitz, M. C. Archer, in preparation).
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11. Light-adapted larval *Rana pipiens* were placed in the urine extract. Test animals were also placed in solutions containing known concentrations of authentic melatonin that had been put through a similar extraction procedure. The melanophore response of each animal was then quantitatively evaluated by microscopic examination, and a calibration curve was plotted relating melanophore response to melatonin concentration. From this curve the melanophore response of test animals exposed to urine extracts could be interpreted in terms of the melatonin contents of the extracts.
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13. In each of the five solvent systems, the R_F of authentic melatonin, and the R_F range within which the melanophorotropic constituent of chromatographically separated urine extract was located, were as follows: (i) methyl acetate, isopropanol, and 25 percent ammonia (45 : 35 : 20), 0.92 and 0.82–1.00; (ii) ethanol, 0.75 and 0.65–0.85; (iii) *n*-butanol, 0.63 and 0.53–0.73; (iv) chloroform, methanol, and acetic acid (93 : 7 : 1), 0.58 and 0.48–0.68; and (v) ethyl acetate, 0.25 and 0–0.35.
14. Some of the studies have previously been described; H. J. Lynch and R. J. Wurtman, paper presented at the symposium, "Advances in fertility regulation through basic research," held at Rockefeller University, New York, 15 and 16 July, sponsored by the Population Council and the Center for Population Research of the National Institute of Child Health and Human Development.
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5-Methyltetrahydrofolic Acid Is Not a Methyl Donor for Biogenic Amines: Enzymatic Formation of Formaldehyde

Abstract. *Contrary to previous reports, 5-methyltetrahydrofolic acid does not mediate the methylation of dopamine to epinine. Instead, this methyl donor is degraded enzymatically to formaldehyde, which condenses with dopamine to form a tetrahydoisoquinoline derivative. The latter has chromatographic characteristics very similar to those of epinine, which likely accounts for the original misidentification of the product.*

It has recently been reported that 5-methyltetrahydrofolate (5-MTHF) is an active methyl donor for biogenic amines such as dopamine, serotonin, and tryptamine (*1, 2*). The reported production of methylated metabolites such as dimethyltryptamine, a highly active psychotomimetic (*3*), has led to speculation regarding the possible implication of 5-MTHF in psychotic disorders such as schizophrenia (*4*).

Because of our interest in biogenic amine methylation reactions (*5*), we attempted to duplicate these findings. In doing this, we found that the compounds formed were chromatographically very similar to, but not identical with, the expected methylated products.

We have shown that in human blood *S*-adenosylmethionine, under certain conditions, can generate formaldehyde by an enzymatic process. Formaldehyde then reacts nonenzymically with indoleamine substrates present in the incubation medium to yield condensation products that are chromatographically very similar to the expected methylated compounds (*6*). The apparent identity, on the basis of our criteria (*6*), of the products formed in 5-MTHF–mediated incubations with those formed in the *S*-adenosylmethionine–mediated blood studies prompted us to investigate the possibility that formaldehyde could be generated from 5-MTHF. Results of our studies on the reactions of indoleamine substrates with 5-MTHF have been described (*7*). We now present evidence that incubation of dopamine with 5-MTHF results not in the production of *N*-methyldopamine (epinine) but rather in the enzymatic formation of formaldehyde which condenses nonenzymatically with dopamine to form 6,7-dihydroxytetrahydroisoquinoline (TIQ) (*8*). We have also found that formaldehyde is generated from 5-MTHF in blood platelet preparations (*9*).

Rat brain enzyme was prepared and incubated essentially by the method of Laduron *et al.* (*10*). Incubation mix-

Table 1. Thin-layer chromatography of ^{14}C-labeled product formed on incubation of [^{14}C]MTHF with dopamine. Solvent system A: phenol, water, and HCl (80 : 20 : 1) (*10*); solvent system B: chloroform, methanol acetic acid, and water (65 : 35 : 10 : 10) for the first dimension, and ethyl acetate, formic acid, and water (70 : 20 : 10) for the second dimension; solvent system C: chloroform, methanol, and acetic acid (93 : 7 : 1). Epinine and TIQ areas on the chromatograms were eluted, and the radioactivity was determined by scintillation counting. The distribution of the radioactivity indicated is relative.

TLC method	Radioactivity distribution (%)	R_F	Width*
One-dimensional cellulose, solvent system A			
Epinine	12	0.75	0.70–0.78
TIQ	88	0.68	0.63–0.72
Dopamine		0.57	0.52–0.62
Two-dimensional silica gel, solvent system B			
Epinine	12	0.58	0.52–0.63†
		0.55	0.50–0.60
TIQ	88	0.50	0.46–0.54
		0.54	0.50–0.58
Dopamine		0.55	0.50–0.60
		0.65	0.61–0.69
One-dimensional silica gel, solvent system C			
Triacetyl epinine	<2	0.65	0.63–0.67
Triacetyl TIQ	>98	0.71	0.69–0.73
Triacetyl dopamine		0.50	0.47–0.53

* Values shown are from representative chromatograms. † First and second values refer to R_F in the first and second dimensions, respectively.

tures contained the following concentrations of reagents in a final volume of 1.0 ml: dopamine, 5 m*M*; potassium phosphate buffer, *p*H 6.5, 0.25 m*M*; [^{14}C]MTHF, 8.3 μM (*11*); EDTA, 5 m*M*; and enzyme. The mixtures were incubated for 2 hours at 37°C and further processed (*10*) through the Dowex-column step. Blanks containing boiled protein were always carried out simultaneously. After lyophilization of the eluate from the Dowex column, the residue was taken up in a small volume of methanol, and portions were subjected to thin-layer chromatography (TLC) on silica gel plates (Merck) (Fig. 1 and Table 1). Separation of mixtures of authentic dopamine, epinine, and TIQ from each other on silica gel was difficult to realize with a large number of solvent systems employed. The best unidimensional separation was obtained with ethyl acetate, formic acid, and water (70 : 20 : 10), but there was considerable overlap of all three compounds. Portions were therefore cochromatographed with either epinine or TIQ. The major peak of radioactivity (Fig. 1) overlaps but does not completely coincide with epinine, whereas it migrates exactly together with TIQ. Although unidimensional chromatography on cellulose, with the solvent system of Laduron *et al.* (*10*) (Table 1, solvent A) gives a better separation of the three compounds, there is still a small amount of overlap of epinine and TIQ. The results in Table 1 shows that little if any epinine is formed, and that the bulk of the radioactivity is found associated with TIQ. Bidimensional TLC on silica gel (Table 1) gave similar results.

Further evidence of the formation of TIQ was obtained from chemical derivatives of the products in the lyophilized eluate from the Dowex column. A sample of the latter to which 50 μg each of unlabeled epinine and TIQ were added was evaporated to dryness, and the residue was taken up in 0.5 ml of 0.04*M* EDTA containing 100 mg of $KHCO_3$ (*p*H 8); acetic anhydride (50 μl) was added. After 10 minutes, the mixture was extracted with chloroform, and a portion was chromatographed. Complete separation of all three triacetylated catechols was obtained (Table 1) (*12*); virtually all of the radioactivity was associated with the triacetylated TIQ and almost none with the epinine derivative.

We have also found that some TIQ is formed in the heat-denatured protein blanks, ranging from 5 to 20 percent of that obtained in a regular sample. Some nonenzymatic breakdown of 5-MTHF to formaldehyde thus appears to occur. Laduron also noted the formation of small amounts of product on incubation of heated protein with [^{14}C]MTHF (*1*).

Still further evidence that [^{14}C]formaldehyde is formed enzymatically from [^{14}C]MTHF was obtained by incuba-

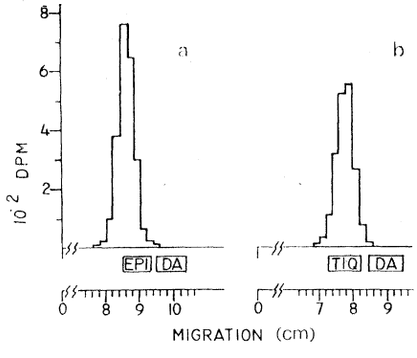

Fig. 1. Distribution of radioactivity on TLC plates of product formed on incubation of dopamine (*DA*) with [^{14}C]MTHF. Plates were developed in a system of ethyl acetate, formic acid, and water (70 : 20 : 10): (a) TLC of Dowex eluate with authentic epinine (*EPI*) standard; (b) TLC with authentic TIQ standard; *DPM*, disintegrations per minute.

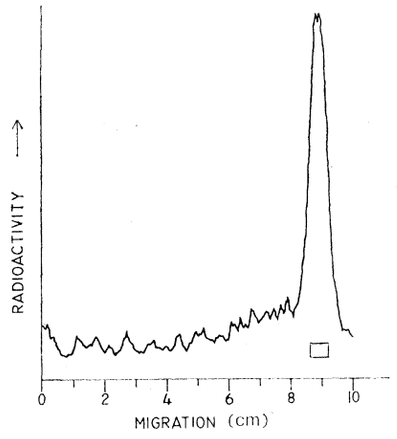

Fig. 2. Radiochromatogram scan of the product formed on incubation of dimedone with [^{14}C]MTHF. Chromatogram was developed in a system of benzene and ethyl acetate (80 : 20). The marker (□) indicates the location of the authentic dimedone-formaldehyde adduct.

tion of a concentrated rat brain preparation containing [^{14}C]MTHF and 5 m*M* dimedone, but no substrate. Dimedone forms a bis-condensation product with aldehydes (*13*). After incubation for 2 hours at 37°C, the product was extracted with chloroform and chromatographed (Fig. 2). The radioactivity was confined to a single major peak which migrated with authentic dimedone-formaldehyde adduct (*13*). A blank with boiled protein gave 15 to 20 percent as much radioactivity in this area as the sample.

Laduron has also reported the *N*-methylation of dopamine to epinine in adrenal medulla, using *S*-adenosylmethionine as methyl donor (*14*). When we repeated this experiment, using either [^{14}C]*S*-adenosylmethionine or [^{14}C]MTHF as methyl donors and subjected the ^{14}C-labeled products formed to the above chromatographic and derivative criteria, we found that [^{14}C]epinine was indeed produced when [^{14}C]*S*-adenosylmethionine was employed. However, only [^{14}C]TIQ was formed when [^{14}C]MTHF was used as the radioactive source. Thus the apparent production of TIQ from 5-MTHF would not appear to be the result of an artifact due to our experimental methodology, since identical procedures were utilized in both cases.

From the evidence presented it appears that 5-MTHF is not a methyl donor for biogenic amines, and that the methylating enzyme which reportedly utilizes it is in actuality an enzyme which catalyzes the formation of formaldehyde from 5-MTHF. Whether

such a process is active in vivo is not yet known.

These results do not necessarily diminish the significance of the initial observation (*1, 2*) that 5-MTHF participates (at least in vitro) in the metabolism of dopamine inasmuch as the isoquinoline derivatives have been shown to be pharmacologically active and may be of biological significance (*7, 15*). However, speculations about the relation of 5-MTHF–derived psychotomimetic substances and schizophrenia require reevaluation in view of the fact that these specific substances are not the ones being produced.

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Chromatin Fragments Resembling *ν* Bodies

Abstract. *Fragments of chromatin resembling the spheroid chromatin units (ν bodies) have been isolated from formaldehyde-fixed and sonicated chicken erythrocyte nuclei. Ultracentrifugal analyses demonstrated that monomer ν bodies have a molecular weight of about 300,000 per particle, exhibit a protein to DNA ratio (by weight) of 1.22:1, and contain a DNA fragment with a molecular weight of approximately 140,000 per ν body.*

Current models of DNA folding into eukaryotic chromosomes include superhelices (*1*) and particles (*2*) as possible first-level structures in a hierarchy of chromatin packaging. The superhelical model of Pardon and Wilkins (*1*) is based on the occurrence of low-angle x-ray reflections from chromatin fibers (at 110, 55, 38, 27, and 22 Å), their semimeridional orientation, and their susceptibility to stretch. The suggestion that chromatin is composed of particulate subunits (*ν* bodies) is based on the observation that isolated nuclei, swollen in water, fixed with formaldehyde, centrifuged onto electron microscope grids, and positively or negatively stained, revealed linear arrays of spherical particles about 70 Å in diameter connected by thin strands about 15 Å wide (*2*).

We have isolated fractions enriched in monomer *ν* bodies and have measured particle and DNA fragment molecular weights. These studies were performed with isolated chicken erythrocyte nuclei because of the convenience and homogeneity of this material. The fact that *ν* bodies have been observed in several other nuclear systems (*2*) suggests that the results presented here have significance to general models of chromatin organization.

Extensive sonication of water-swollen and formaldehyde-fixed chicken erythrocyte nuclei did not destroy the *ν* bodies but did obliterate the fibrillar arrangement of particles (*2*). The disrupted chromatin containing particles furnished the starting material for fractionation by sucrose gradient ultracentrifugation (Fig. 1A). Electron microscopic analyses of successive fractions from such ultracentrifugation ex-

Table 1. Molecular parameters of monomer *ν* bodies and of *ν*-DNA fragments.

Pools*	*ν* bodies			*ν*-DNA	
	Molecular weight†	$\rho_{CsCl,\ 25°C}$	Ratio of protein to DNA‡	Molecular weight§	$\rho_{CsCl,\ 25°C}$
A	296,000	1.414	1.22	141,000	1.691
B	268,000	1.416	1.19	141,000	1.689
C	330,000	1.413	1.23	142,000	1.690
Average‖ ± S.E.	295,000 ± 13,000	1.414 ± 0.001	1.22 ± 0.01	141,000 ± 2,000	1.690 ± 0.001

* Analyses of positively stained preparations of pools A, B, and C with the electron microscope yielded average monomer particle diameters of 84 ± 6 Å, in reasonable agreement with the range of 60 to 80 Å for monomer *ν* bodies noted previously (*2*). The percentage of monomer particles was also estimated from micrographs for pools A, B, and C to be approximately 64, 50, and 74 percent, respectively. We believe that microscopic estimation of purity of particles tends to minimize the proportion of monomer *ν* bodies, probably due to aggregation of single particles on the electron microscope grid. Sedimentation velocity and sedimentation equilibrium centrifugation analyses of the pools in the guanidine-HCl solvents gave no indications of large proportions of multimers. † Molecular weights of the pools enriched with monomer *ν* bodies were determined by sedimentation equilibrium centrifugation in guanidine-HCl solutions ranging from 2 to 6.5*M*, buffered with 0.02*M* tris (*p*H 7.0). Partial specific volume ($\bar{\nu}$) was calculated from a plot of $(\eta/\eta_0)S$ versus ρ (where η is viscosity; S, sedimentation coefficient; and ρ, density) over the same range of solvent molarities (*11*). The plot was linear over the range of 2 to 6.5*M* guanidine-HCl and yielded a $\bar{\nu}$ of 0.747 ± 0.011 (ml/g), calculated by extrapolation of the value of ρ corresponding to zero sedimentation rate. Data from the sedimentation equilibrium experiments usually yielded linear plots of log concentration versus r^2 (where r is the distance from the center of rotation). In 4.25*M* guanidine-HCl, equilibrium was usually achieved by 86 hours at 6000 rev/min, 20.7°C. Relative viscosities and densities of the guanidine-HCl solutions were calculated from empirical equations (*12*), with molarities and weight fractions of each solution determined by refractometry (*13*). ‡ The ratio of protein to DNA (by weight) was calculated from $\rho_{CsCl,\ 25°C}$ as described by Brutlag *et al.* (*14*), in which we used ρ of chicken DNA as 1.694 (our data) and ρ of histones, 1.245 (*14*). § DNA fragments derived from various pools of *ν* bodies after extensive digestion with Pronase. Samples of pools A, B, and C were dispersed in 0.35*M* of the sodium salt of ethylenediaminetetraacetic acid, 0.7 percent sodium dodecylsulfate (SDS), and 7 m*M* tris (*p*H 9.5) and heated for 30 minutes at 65°C; Pronase was added to a concentration of 500 μg/ml, following a procedure developed by Kavenoff and Zimm (*15*). Digestion was allowed to proceed at 50°C for 6½ hours, and the resulting digest was dialyzed extensively against SSC buffer [0.15*M* NaCl and 0.015*M* sodium citrate (*p*H 7.0)] at room temperature. We measured the molecular weights by sedimentation equilibrium ultracentrifugation in SSC buffer, assuming a $\bar{\nu}_{DNA}$ of 0.503 (*16*). ‖ Averages and standard errors (S.E.) are of all reliable determinations, *not* averages of the average value for each pool. The data presented in this table were calculated on an Olivetti Programma 101 calculator with the use of the programs of Trautman (*17*). The molecular weights calculated from sedimentation equilibrium ultracentrifugation, in which the programs of Trautman were used, represent the weight-average molecular weight at the midpoint of r^2 in the sample solution.