Intranuclear Localization of Mercury in vivo

Abstract. *Purified nuclei isolated from mice challenged with nonlethal levels of mercury chloride (10^{-3}M) in drinking water for 4 to 7 weeks (experimental) and from animals given deionized water (control) were fractionated and the subsequent fractions were analyzed for mercury by flameless atomic absorption. Control (active) euchromatin contained 1.75 ± 0.53 micrograms of mercury per milligram of DNA. There was a 12- to 15-fold enrichment of mercury in the euchromatin fraction of challenged animals. Mercury was not detected in control (inactive) heterochromatin, and only trace levels (parts per billion) appeared in experimental heterochromatin. It seems likely that mercury can be incorporated into chromatin as a metal-protein complex, but the possibility of protein-mercury-DNA or mercury-DNA complexes within euchromatin cannot be excluded.*

Although nonhistone chromosomal proteins are believed to play a key role in the control of gene expression, the mechanisms by which these proteins participate in the specific regulation of gene transcription in nucleated cells remain to be resolved (*1*). The fact that chromatin, the complex structure of the eukaryotic genome, contains metals along with DNA, RNA, histone, and nonhistone proteins could be important in understanding how nonhistone proteins interact with the genome. Both copper (*2*) and mercury (*3*) are among the metals present in native chromatin. Fractionation of chromatin into "unique" species of (repressed) heterochromatin and (active) euchromatin (*4*) and the subsequent analysis of each species has revealed copper in both heterochromatin and euchromatin in control materials, with a pronounced enrichment of copper in the heterochromatin fraction of challenged mice (*2*). We now investigate mercury and find that the pattern of intranuclear localization and accumulation is strikingly different from copper: mercury appears to be confined to the euchromatin component in both control and metal-challenged animals.

Experiments were carried out in male Swiss-Webster strain white mice obtained within 4 weeks of birth from commercial sources and separated into cages containing six animals each. All mice had free access to Purina Lab Chow throughout the study. Control animals were given deionized drinking water and experimental animals received deionized drinking water containing 1 m*M* $HgCl_2$. After 4 to 7 weeks animals were anesthetized and exsanguinated by cardiac puncture. Livers were removed and placed in physiological saline at 4°C. Liver nuclei were isolated as described previously (*3*) according to a modification of the method of Chauveau (*5*), and heterochromatin and euchromatin were obtained by the procedure of Yasmineh and Yunis (*4*). Deoxyribonucleic acid was assayed by absorbance at 260 nm after the addition of 0.1 ml of chromatin to 2 ml of 1 percent sodium dodecyl sulfate, in 0.01*M* tris-buffered 5 m*M* ethylenediaminetetraacetic acid, *p*H 8.0, standardized such that 50 μg of DNA per milliliter gives an absorbance of 1.0 at 260 nm. Mercury was analyzed by flameless atomic absorption described previously (*3*). Samples were digested prior to mercury analysis by refluxing for 2 hours in a concentrated nitric acid–hydrochloric acid (1 : 1) mixture, cooled, diluted to either 25 or 50 ml, and stored in plastic containers (Nalgene) for less than 48 hours before mercury determinations were made.

We have previously reported mercury present at readily detectable levels in control nuclei and control chromatin (*3*); additionally, that material obtained from animals challenged with a constant level of mercury showed a 20-fold enrichment of metal (relative to control) in nuclei and a 10-fold accumulation (not removed by gel filtration) in chromatin (*3*). We now report a similar enrichment pattern in the euchromatin component of chromatin. With 1.75 μg of mercury per milligram of DNA used as the average of the three controls reported in Table 1, there is a 12- to 15-fold enrichment in euchromatin isolated from mercury-challenged animals after 4 to 7 weeks of treatment. The fact that there was no measurable mercury in the other fractions is of special interest. In an attempt to detect the metal in these fractions, larger quantities of sample were digested. When the sample volume of heterochromatin was four times that of euchromatin (in mercury-treated animals), only a trace of the metal was detected in heterochromatin. There was

Table 1. Mercury levels in chromatin fractions. Following the procedure of Yasmineh and Yunis (*4*), washed nuclei were suspended in 0.25*M* cation-free sucrose (to give an optical density 425_{nm} of 1.0 unit) and allowed to swell for 20 minutes at 4°C. Forty-milliliter samples were cooled in an ice bath and each was sonicated for 1.0 minute at 80 amp on 15-second intervals with the Branson 5-75 Sonifier. The sonicates were spun first at 500*g* for 10.0 minutes to remove unbroken nuclei or aggregates, and the heterochromatin pellet was obtained by recentrifugation at 3500*g* for 20 minutes. The supernatant was spun at 78,000*g* for 1 hour, yielding a compact, translucent pellet of intermediate-type chromatin. The supernatant was made 0.14*M* in NaCl and added to two volumes of ethanol to precipitate out a fluffy white euchromatin pellet. All fractions were suspended and stored frozen in 0.01*M* tris buffer, *p*H 8.0, after routine light microscopic examination of air-dried, Giemsa-stained samples. Abbreviations: Het, heterochromatin; Int, intermediate; Euch, euchromatin.

Experiment	Animals (No.)	Fraction	Mercury (μg/mg DNA)
		Control	
1	31	Het	*
		Int	*
		Euch	1.16
2	15	Het	*
		Int	*
		Euch	2.15
3	15	Het	*
		Int	*
		Euch	1.98
		Mean†	1.75 ± 0.53
		Experimental‡	
4§	20	Het	*
		Int	*
		Euch	21.80
5¶	20	Het	0.02
		Int	0.03
		Euch	26.50
		Mean†	24.15 ± 3.33

* Less than 0.5×10^{-3}. † Values are means, for euchromatin, of experiments shown, ± S.D. ‡ Experimental was significantly enriched over control (*t*-test, $P < .01$). § Animals were treated with 1 m*M* $HgCl_2$ for 4 weeks. ¶ Animals were treated with 1 m*M* $HgCl_2$ for 7 weeks.

Table 2. Metal binding following treatment with exogenous mercury in vitro. Mercury (approximately 5 μg per milligram of DNA) was added to 1.5 ml of sample and kept at 37°C for 30 minutes before mercury and DNA analyses and gel filtration. Each value represents mean ± S.D.

Sample*	Mercury (μg/mg DNA)	
	Before gel filtration†	After gel filtration†
Heterochromatin	3.56 ± 0.05	1.67 ± 0.39
Euchromatin‡	3.29 ± 0.16	11.71 ± 0.29

* Obtained from control animals of experiment 2 (Table 1). † One-half milliliter of the sample to which mercury (approximately 5 μg/mg DNA) had been added was added to a Sephadex G-25 column (19 by 1 cm) equilibrated with 1 : 100 SSC (0.14*M* NaCl, 0.014*M* sodium citrate, *p*H 7.0) containing 0.163 ppm of mercury chloride. A 1.5-ml aliquot which followed the void volume (determined by Blue Dextran 2000) and which contained all of the chromatin fraction (determined by A_{260} profiles) was collected and analyzed for mercury and DNA. ‡ Mercury binding to euchromatin was significantly greater than to heterochromatin (*t*-test, $P < .01$).

a marked elevation of mercury in euchromatin compared to the level in heterochromatin (Table 1). The in vitro experiment (Table 2) further supports the specificity of mercury-euchromatin binding.

Chanda and Cherian have observed a 35- to 45-fold incorporation (relative to other fractions) of labeled mercury into a nuclear protein (nonhistone) fraction (*6*). In their experiment using kidney nuclei obtained 5 to 6 hours after a single injection of mercury, no significant amount of metal accumulated in the other fractions.

When total histones are extracted from native heterochromatin and euchromatin complexes are prepared in parallel from a single tissue sample, the histone-DNA ratios are only slightly different between the two types of chromatin (*7*). However, nuclear constituents such as nonhistone residual protein, RNA, phosphoprotein, and phospholipid were found to be present in a significant excess within euchromatin as compared to (repressed) heterochromatin; in particular, an excess of nonhistone residual protein is present within (active) euchromatin (*7*). On the basis of Chanda and Cherian's findings, in which mercury is bound largely to insoluble nonhistone protein, and of our present experiments, in which mercury is bound to euchromatin, in addition to our experiments reported previously (*3*), it seems likely that mercury is bound in a metal-protein (most likely nonhistone) complex within the euchromatin component of chromatin. The possibility of protein-metal-DNA or metal-DNA complexes, however, cannot be ruled out. Thus, in contrast to copper, mercury appears to be largely confined to euchromatin. The significance of finding mercury at rather constant levels (1.75 ± 0.53 μg per milligram of DNA) in control euchromatin and the accumulation of the metal in this fraction in challenged animals is not known. However, in view of growing evidence that nonhistone proteins are regulators of gene expression in eukaryotic cells (*1*), nuclear constituents which associate with these proteins are of interest and their localization within chromatin is a step toward the elucidation of possible functional or structural roles, or both, for the nuclear components.

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Histaminergic Pathway in Rat Brain Evidenced by Lesions of the Medial Forebrain Bundle

In spite of the marked biological activities of histamine and its presence in most tissues, its physiological function is still a matter of controversy. However, data have been accumulating which suggest an analogous role of brain histamine to that of other biogenic amines, such as the catecholamines and serotonin.

Histamine is unevenly distributed in the central nervous system, with highest concentrations in gray matter (*1*). Subcellular fractionation of brain homogenates reveals that a significant portion of the amine as well as of its specific synthesizing enzyme (L-histidine decarboxylase) (*2*) is apparently localized in nerve terminals (*3–5*). Neuronal receptors to histamine are apparently present in the brain, since the exogenous amine has been reported to induce electrical responses (*6*), behavioral effects (*7, 8*), and activation of cyclic adenosine monophosphate formation (*9*); in all cases, these effects were antagonized by classical antihistamines.

Taken together, these observations suggest that histamine is a central neurotransmitter; however, because the lack of a suitable histochemical method, the localization and even the presence of specific histaminergic neuronal tracts in the central nervous system have yet to be demonstrated.

We have now found that diencephalic lesions involving the medial forebrain bundle (MFB) induce a progressive decrease in both the histamine concentration and L-histidine decarboxylase activity of the telencephalon. This decrease occurs in a manner suggesting that it is due to the anterograde degeneration of histaminergic fibers present in this tract, which is already known to comprise the bulk of the ascending noradrenergic and serotoninergic fibers (*10*).

Sprague-Dawley male rats (200 to 250 g) were used. Brain lesions intended to interrupt the MFB were made at the lateral hypothalamic area (*11*). Their effect was checked by histological examination of serial sections of brain from some animals of each group and also by determination of noradrenaline and serotonin in the cortex (*12*). Both these noradrenaline and serotonin concentrations were decreased after 12 days by 64 ± 2 percent and 63 ± 4 percent, respectively, values in agreement with previous reports (*13*). After various time intervals animals were decapitated and the regions of the brain, dissected according to Glowinski and Iversen (*14*), were homogenized in five to ten volumes of cold phosphate buffer at *p*H 7.4. L-Histidine decarboxylase activity was evaluated by a radiochromatographic assay consisting in the determination of [^{3}H]histamine formed from a low concentration of highly labeled [^{3}H]histidine (*3*). The incubation mixture (70 μl) consisted of 5 μc (about 0.1 nmole) of [^{3}H]histidine, histamine dihydrochloride ($10^{-4}M$), pyridoxal 5′-phosphate ($10^{-5}M$), 50 μl of tissue extracts and phosphate buffer, *p*H 7.4 ($0.05M$). After a 60-minute incubation period the [^{3}H]histamine was isolated on an Amberlite CG 50 column (*15*).

For determination of histamine, perchloric acid was added to the homogenate to a final concentration of $0.4M$, and the amine was estimated spectrofluorometrically after isolation by ion-exchange chromatography (*16*).

Preliminary experiments indicated that unilateral lesions involving the MFB resulted in an ipsilateral lowering of L-histidine decarboxylase activity in the forebrain. In order to check whether the time course of this reduction was compatible with that of neuronal degeneration, enzyme activity and the histamine concentration in the cortex were measured at intervals after