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.

SARA E. BRYAN, CHARLES LAMBERT
KENNETH J. HARDY, SAM SIMONS
Department of Biological Sciences, University of New Orleans, New Orleans, Louisiana 70122*

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* Formerly Louisiana State University in New Orleans.

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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

unilateral placement of the lesions (Table 1). The L-histidine decarboxylase activity in the ipsilateral cortex was significantly reduced after 2 days ($P < .01$) and maximally reduced (50 percent) by 8 days. Thus the half-life of the enzyme decline was slightly more than 2 days, a value similar to that reported for the reduction of tryptophan hydroxylase after the midbrain raphe was lesioned (*17*). Histamine concentrations (Table 1) also decreased significantly but to a somewhat lesser extent: at 8 days they were maximally reduced by 30 percent. This moderate reduction, relative to that observed for noradrenaline or serotonin, might explain previous negative results (*18*).

To obtain more information on the origin and termination of the histaminergic pathway whose existence was suggested by the preceding experiments, the regional distribution of L-histidine decarboxylase activity was determined 12 days following the lesion.

Although enzyme activities were not significantly altered in caudal regions (medulla, pons, cerebellum, and midbrain), in all regions rostral to the lesion, including the anterior hypothalamus, significant reductions in enzyme activity relative to the nonlesioned side were found (Table 2). By contrast, the enzyme activities in the intact side did not differ significantly from those found in a group of six nonoperated animals, indicating that the telencephalic degenerations were restricted to the lesioned side. Therefore our data show that lesions intercepting the MFB result in alterations in the levels of histamine and its forming enzyme in rat brain.

Both the time course of the alteration—compatible with a process of Wallerian degeneration—and its regional distribution (enzyme activity decreased in the whole forebrain but unchanged caudally to the lesion) suggest the presence of histamine in an ascending neuronal fiber system. This system resembles the MFB as it seems to emanate from the brainstem or mesencephalon, to pass through the lateral hypothalamic area, and then to spread ipsilaterally in the whole telencephalon (*19*).

This new ascending aminergic system, as suggested by the widespread distribution of its terminals in all parts of telencephalon, may have functional roles similar to those ascribed to the monoamines—such as in the control of states of sleep and wakefulness or in self-stimulation behavior. Other observations indirectly support this hypothesis: the sedative properties, including somnolence, of most antihistamines in therapeutic doses (*20*), the marked decrease in histamine turnover induced in rat brain by several hypnotics including barbiturates (*21*), and the effects of histamine and its amino acid precursor on self-stimulation (*8*), a behavior whose main anatomical substrate is known to be the MFB. The synergistic interaction between histamine and noradrenaline in stimulation of cyclic AMP formation in cortex (*22*) might indicate a relationship of importance in the control of the cortical cells upon which several kinds of amine-containing fibers from the MFB project.

However, as relatively high levels of histamine and L-histidine decarboxylase activity remain after degeneration of the MFB, this amine may be present elsewhere in brain tissues (*23*).

Table 1. Time course of decreases in cortical L-histidine decarboxylase activity and histamine following a lesion in the lateral hypothalamic area. Results are expressed as the mean (± the standard error of the mean) percent decrease in the lesioned side as compared to the control side of the same animal. The means (± S.E.M.) in the control side were 1250 ± 60 disintegrations per minute (dpm) per milligram per hour for L-histidine decarboxylase activity, and 47 ± 2 ng g^{-1} for histamine. They did not differ significantly either from sham-operated or from control rats. Groups of 5 to 14 animals were used.

Post-operative day	Change from control side	
	L-Histidine decarboxylase (%)	Histamine (%)
2	− 16 ± 3	
4	− 37 ± 4	− 20 ± 7
8	− 49 ± 5	− 32 ± 4
12	− 51 ± 4	− 30 ± 4
21	− 49 ± 4	− 28 ± 2

Table 2. Effects of a lateral hypothalamic lesion on L-histidine decarboxylase activity in various regions of rat brain. Results were obtained from six animals killed 12 days after a unilateral section was performed at the lateral hypothalamic area. Values for L-histidine decarboxylase activity are expressed as the means. The percentage changes from control are the means ± S.E.M. obtained by averaging the percentage changes for each animal.

Region	L-Histidine decarboxylase activity (dpm μg^{-1} $hour^{-1}$)		Change from control side (%)
	Control side	Lesion side	
Cortex	1.01	0.51	− 47 ± 7*
Striatum	1.20	0.77	− 36 ± 12†
Hippocampus	0.40	0.16	− 60 ± 4*
Hypothalamus (anterior)‡	5.19	3.41	− 28 ± 11†
Midbrain	2.03	1.89	− 6 ± 4
Pons	0.79	0.64	− 19 ± 10
Medulla	0.26	0.28	+ 4 ± 13
Cerebellum	0.17	0.21	+ 29 ± 26

* $P < .001$. † $P < .05$. ‡ The anterior hypothalamus refers to the part rostral to the section.

MONIQUE GARBARG
GILLES BARBIN
JEAN FEGER
JEAN-CHARLES SCHWARTZ
Unité de Neurobiologie,
Institut National de la Santé
et de la Recherche Médicale,
2 ter Rue d'Alésia, 75014 Paris

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Interferon Induction: Tool for Establishing Interactions among Homopolyribonucleotides

Abstract. *Hitherto unrecognized interactions between homopolyribonucleotides and complexes thereof are suggested by interferon induction data obtained in a highly sensitive assay system of primary rabbit kidney cell cultures superinduced by metabolic inhibitors.*

Interferon induction can be considered a stringent biological test for the double-strandedness of polyribonucleotides, since double-stranded RNA's are markedly more effective as interferon inducers than their single- and triple-stranded counterparts (*1–4*). Primary rabbit kidney cell cultures are among the most sensitive cell types for assaying the interferon-inducing capacity and antiviral activity of double-stranded RNA's (*5, 6*), and their responsiveness is even enhanced when the cells are treated with metabolic inhibitors, such as cycloheximide and actinomycin D, sometime after their exposure to the double-stranded RNA (*7*). This paradoxical enhancement of interferon production by judicious treatment with metabolic inhibitors has been referred to as "superinduction," a term originally coined for the increase of tyrosine aminotransferase in hydrocortisone-induced cells upon actinomycin D treatment (*8*). In this report, we demonstrate that this sensitive assay system can be employed to identify the occurrence of novel polynucleotide interactions and to gain insight into the nature of the resultant product or products.

In primary rabbit kidney cell cultures superinduced with cycloheximide and actinomycin D, the homopolymers (*9*) polyadenylic acid [poly(A)] and polyuridylic acid [poly(U)], on the one hand, and polyinosinic acid [poly(I)] and polycytidylic acid [(poly(C)], on the other hand, proved unable to reverse the interferon-inducing capacity of the respective duplexes poly(A) · poly(U) and poly(I) · poly(C) when the duplexes were applied to the cells 1 hour later (*4*).

It was concluded from these data that single-stranded homopolymers did not prevent the interaction of the active homopolymer duplexes with the postulated (*2*) cellular receptor site (or sites) for interferon induction. However, single homopolymers were not invariably inactive in reducing the interferon-inducing capacity of the active duplexes; depending on the kind of homopolymer that was added first and on the kind of the homopolymer complex that was added second, the interferon-inducing activity of the latter was depressed, enhanced, or unaffected (Table 1). For example, poly(I) and poly(dUz) [poly(2′-azido-2′-deoxyuridylic acid)] (*10, 11*) caused a significant reduction of the interferon-inducing capacity of poly(A) · poly(U) and poly(A) · poly(rT) (polyribothymidylic acid) applied to the cells 1 hour after the homopolymers were applied. However, poly(I) and poly(dUz) did not affect the activity of poly(I) · poly(C). Poly(C) did not influence the interferon-inducing activity of poly(A) · poly(U) or poly(A) · poly(rT) but boosted the interferon response to poly(A) · poly(I) (a triple-stranded polymer) up to the level generally observed for poly(I) · poly(C). The interferon-inducing activity of poly(A) · poly(U) was not only reversed by poly(I) and poly(dUz) but also by analogous homopolymers, such as poly(X) (*12*) and poly(dUf) [poly(2′-fluorodeoxyuridylic acid)] (*13*) (data not shown).

With most systems studied, similar shifts in interferon production were noted regardless of whether the single homopolynucleotide (*a*) was added to the cells before the homopolynucleotide complex (*b*) (that is, $a \rightarrow b$), after ($b \rightarrow a$), or together ($a + b$). These data suggest that both in the test tube and at the cellular level homopolynucleotide complexes, such as poly(A) · poly(U), must be equally susceptible to fundamental structural transitions. That poly(I) may cause such alterations at the cellular level is no surprise, since poly(I) has been shown previously to form an active complex with poly(C) after the polymers had been added to cells separately in an interval of one to several hours (*6*). Although poly(U) reduced the activity of poly(A) · poly(U) when mixed with, or applied to, the cells after poly(A) · poly(U), it failed to do so when applied to the cells before poly(A) · poly(U). In marked contrast, poly(dUz) reversed the activity of poly(A) · poly(U) when it was applied to the cells before the duplex. The differential behavior of poly(U) and poly(dUz) may be accounted for by differences in susceptibility to degradation by nucleases. Poly(U) is very susceptible, whereas poly(dUz) is not (*10*). Poly(U) may have been degraded by cellular nucleases before it could be reached by poly(A) · poly(U).

Several lines of evidence indicate that the shifts in interferon production by the systems depicted in Table 1 originate from a specific interaction between the single homopolynucleotide and the