goes in situ thermal denaturation more readily than DNA in interphase cells. This suggests that tightly condensed chromatin is more easily disrupted by thermal stress (*8*). It has also been observed that DNA in pyknotic (hyperchromatic) nuclei of somatic cells denatures in a fashion similar to that of DNA in sperm from subfertile mammals (*8*). However, chromatin in mature mammalian sperm is even more condensed than that in interphase or mitotic somatic cells, yet the DNA in fertile sperm is not readily denatured by heat. It should be emphasized that the nature of condensed chromatin in metaphase chromosomes and mature sperm is very different, the latter having a different protein composition and substantial disulfide bonding.

Although the mechanism behind the differential sensitivity of sperm chromatin to thermal denaturation is not known, this method offers numerous advantages for studying fertility problems. The analysis is objective, and cell selection is unbiased. Statistical significance is easily attainable, since 10^5 cells can be analyzed in less than 2 minutes. We expect this assay to have application in many research areas, including animal husbandry, human infertility, and environmental and public health.

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3. Semen samples, either frozen and thawed, or obtained fresh were diluted ten times in 0.01*M* tris, 0.15*M* NaCl, and 0.001*M* EDTA (*p*H 7.4) and washed three times by centrifuging (2500*g*) through the buffer. The sperm was resuspended in 2.6 ml of the same solution and sonicated for 1 minute with a Branson Sonifier (model 185, Branson Sonic Power). The sonicate was mixed with one-third of an equal volume of 60 percent (by weight) sucrose in 0.01*M* tris-HCl (*p*H 7.4) and 2 m*M* EDTA, layered on 8 ml of the sucrose buffer solution, and centrifuged at 37,000*g* for 60 minutes (*9*). After aspiration of the supernatant, the pellet was resuspended in 1 ml of 0.15*M* NaCl, 5 m*M* $MgCl_2$, and 20 m*M* tris-HCl (*p*H 7.4) and then forcefully pipetted into 9 ml of a 1:1 mixture of 70 percent ethanol and acetone. All of the above operations were done at 4°C. After overnight fixation at 4°C, the cells were pelleted and resuspended in 2 m*M* cacodylate, 10^4*M* EDTA, and 40 percent (by volume) ethanol (*p*H 6.0). Portions (0.5 ml) containing about 2×10^5 cells per milliliter were either not heated or heated at 100°C for 5 minutes, admixed with 2 ml of staining solution consisting of 0.15*M* NaCl, 5 m*M* $MgCl_2$, 20 m*M* tris-HCl (*p*H 7.4), and 2.67×10^{-5}*M* AO (*2*). After 1 minute the fluorescence of individual nuclei was measured with an FC 200 Cytofluorograf (Ortho Diagnostic Instruments) interfaced with a Data General minicomputer.
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High-Affinity [^{3}H]Imipramine Binding in Rat Hypothalamus: Association with Uptake of Serotonin but Not of Norepinephrine

Abstract. *Inhibition of the binding of [^{3}H]imipramine and inhibition of the uptake of [^{3}H]serotonin and [^{3}H]norepinephrine by a series of antidepressants and other drugs were studied in the rat hypothalamus. No correlation was found between the potencies of these drugs for the inhibition of [^{3}H]imipramine binding and the inhibition of [^{3}H]norepinephrine uptake. There was, however, a highly significant correlation between the potencies of these drugs for the inhibition of [^{3}H]imipramine binding and the inhibition of [^{3}H]serotonin uptake. These results suggest that high-affinity [^{3}H]imipramine binding might be associated with the mechanism of serotonin uptake in the brain.*

The monoamine hypothesis (*1*) is not an entirely satisfactory explanation of the mode of action of tricyclic antidepressants (*2*). The recent demonstration that there are specific high-affinity binding sites for the tricyclic antidepressant imipramine in the brain (*3*) and platelets (*4*) of various species, including man, has provided new means of studying their mechanisms of action.

Tricyclic antidepressant drugs inhibit the binding of ^{3}H-labeled imipramine in the rank order of their clinical potencies (*5*). The stereoselectivity of the imipramine binding site has been recently demonstrated by use of isomers of the 10-hydroxy derivatives of amitriptyline and nortriptyline and isomers of zimelidine and norzimelidine (*6*). A study of the potencies of a wide range of drugs that inhibit the binding of [^{3}H]imipramine has demonstrated that the site of imipramine binding is distinct from those of the known neurotransmitter receptors. However, since imipramine competitively inhibits [^{3}H]serotonin uptake (*7*), and since several other inhibitors of the serotonin uptake, such as fluoxetine and nitalapram (Lu-10171), also have relatively high affinities for [^{3}H]imipramine binding sites, an association of imipramine binding with the mechanism for serotonin uptake could not be ruled out (*3*).

We compared the potencies of a series of antidepressants and other compounds

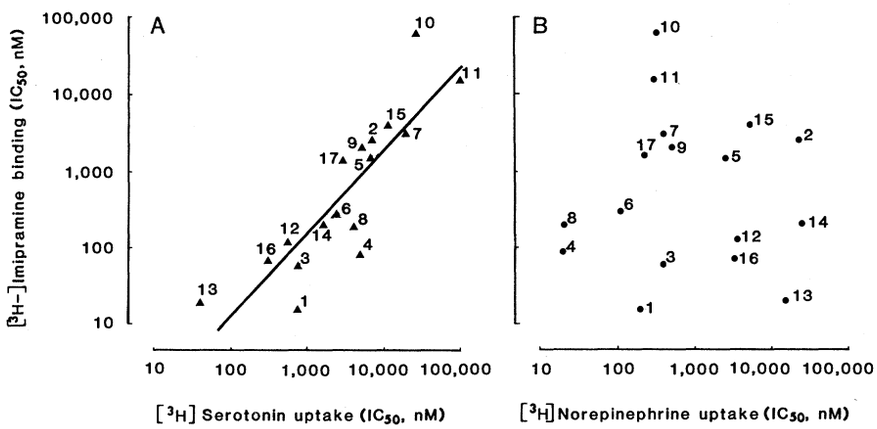

Fig. 1. Comparison of the inhibition of [^{3}H]imipramine binding with the inhibition of neuronal uptake of serotonin and norepinephrine by various drugs. The IC_{50} values (taken from Table 1) for the inhibition of [^{3}H]imipramine binding are compared with the IC_{50} values for the inhibition of neuronal uptake of (A) serotonin and (B) norepinephrine. Each point represents a different drug identified by a number in Table 1. The regression line in (A) is fitted by the method of least squares.

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Table 1. Inhibition of [^{3}H]imipramine binding and the neuronal uptake of [^{3}H]serotonin and [^{3}H]norepinephrine. The IC_{50} values are the concentrations of drugs required to inhibit by 50 percent the specific binding of [^{3}H]imipramine, the neuronal uptake of [^{3}H]serotonin, or the neuronal uptake of [^{3}H]norepinephrine. The active uptake of [^{3}H]norepinephrine was 5.80 ± 0.21 nCi per milligram of tissue in 15 minutes (N = 31) and of [^{3}H]serotonin was 4.80 ± 0.20 nCi per milligram of tissue in 5 minutes (N = 29). The ratios of tissue to medium were 9.40 ± 0.33 (N = 31) for norepinephrine and 9.14 ± 0.38 (N = 29) for serotonin.

Drug	IC_{50}, nM		
	[^{3}H]Imipramine binding	[^{3}H]Serotonin uptake	[^{3}H]Norepinephrine uptake
1. Imipramine	15	760	200
2. IMIH	2,500	7,030	23,280
3. 2-Hydroxyimipramine	60	760	380
4. Desipramine	90	4,780	20
5. DMIH	1,500	6,840	2,500
6. Nortriptyline	300	2,400	110
7. 10-Hydroxynortriptyline (z)	3,000	19,450	400
8. Nisoxetine	200	4,030	20
9. Cocaine	2,000	5,270	520
10. *d*-Amphetamine	60,000	26,280	320
11. Metaraminol	15,000	100,000	300
12. Fluoxetine	120	560	3,520
13. Nitalapram (Lu-10171)	20	40	15,370
14. Zimelidine (z)	200	1,640	25,260
15. Zimelidine (e)	4,000	11,850	5,040
16. Norzimelidine (z)	70	320	3,340
17. Norzimelidine (e)	1,500	2,980	220

for their inhibition of the uptake of [^{3}H]-serotonin and [^{3}H]norepinephrine and their inhibition of specific [^{3}H]imipramine binding in rat hypothalamus. We now report a significant correlation between the inhibition of [^{3}H]imipramine binding and the inhibition of the uptake of [^{3}H]serotonin but not of [^{3}H]norepinephrine.

Inhibition of [^{3}H]amine uptake was measured in 0.4-mm slices of rat hypothalamus by a modification of the method of Shaskan and Snyder (*8*). The tissue samples (two slices, 8.2 ± 0.3 mg, wet weight) were incubated in Krebs solution at 37°C with various concentrations of the test drugs. After 10 minutes, the [^{3}H]amine ([^{3}H]serotonin, 12.0 Ci/mmole, Radiochemical Centre, or *dl*-[^{3}H]norepinephrine, 15.2 Ci/mmole, New England Nuclear) was added at a final concentration of 50 nM in a total incubation volume of 2 ml. After 5 minutes of incubation for [^{3}H]serotonin and 15 minutes for [^{3}H]norepinephrine, the uptake was terminated by filtration through Millipore filters (type RA, pore size, 1.2 μm in diameter) and washing twice with 2 ml of cold Krebs solution. A parallel experiment was carried out at 0°C as a control for passive diffusion. The radioactivity in the media and in the tissues was determined by liquid scintillation spectrometry. The inhibition of uptake was calculated according to the formula

$$\text{Percent inhibition of uptake} = [(R_c - R_t)/(R_c - R_0)] \times 100$$

where R_c is the ratio of tissue to medium for the control tissue, R_t is the ratio of tissue to medium for the tissue incubated with the test compound, and R_0 is the ratio of tissue to medium for the control tissue at 0°C.

[^{3}H]Imipramine binding was measured in washed membrane preparations of rat hypothalamus as described in (*3*). The membrane suspension [30 mg of original wet tissue weight per milliliter (approximately 3 mg of protein per milliliter)] was incubated with 2 nM [^{3}H]imipramine (29.8 Ci/mmole, New England Nuclear) with or without the test drug for 60 minutes at 0°C. After incubation, 100 μl was rapidly diluted in 5 ml of ice-cold buffer and immediately filtered through Whatman GF/F glass fiber filters. The filters were washed three times with 5 ml of ice-cold buffer and dried; radioactivity was determined by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding in the presence and absence of 100 μM desipramine and represented 60 to 70 percent of the total binding.

To compare the effects on binding and monoamine uptake of a series of compounds with both major and minor structural differences, we studied selected tricyclic antidepressant derivatives together with a series of monoamine uptake inhibitors having different selectivities for the norepinephrine and serotonin systems. The drugs tested (Table 1) included the derivative of imipramine in which one of the aromatic rings is fully reduced to a cyclohexane (IMIH) (*9*) and the equivalent derivative of desipramine (DMIH) (*9*), the 2-hydroxy metabolite of imipramine, and the 10-hydroxy metabolite of nortriptyline. In addition, the stereoisomers of the new atypical antidepressant drug, zimelidine, and its active metabolite, norzimelidine, (*10*) were also tested.

Values for the inhibition of [^{3}H]imipramine binding by the various drugs were measured in the rat hypothalamus (Table 1) and are very similar to values obtained from the rat cortex (*3*) and human platelets (*4*). The values for inhibition of the neuronal uptake of [^{3}H]serotonin and [^{3}H]norepinephrine measured in the rat hypothalamus (Table 1) were also similar to those previously reported (*8, 10, 11*).

When the values for the inhibition of [^{3}H]imipramine binding and the inhibition of the uptake of [^{3}H]norepinephrine are compared (Table 1) there is no significant correlation between the two measures (Fig. 1B) (r = .012, N = 17, P > .25). The correlation between the inhibition of [^{3}H]imipramine binding and the inhibition of [^{3}H]serotonin uptake (Table 1) is, however, highly significant (Fig. 1A) (r = .862, N = 17, P < .001).

The correlation for the uptake of [^{3}H]serotonin is still valid if the pharmacological subgroups are studied separately (Fig. 1A). If the tricyclic antidepressants and their derivatives (r = .881, N = 7, P < .01) or the monoamine uptake inhibitors (r = .908, N = 10, P < .001) are considered by themselves, the correlations are still significant.

The lack of correlation between the inhibition of [^{3}H]norepinephrine uptake and inhibition of [^{3}H]imipramine binding suggests that there is no direct association between the [^{3}H]imipramine binding site and the mechanism for norepinephrine uptake. Although the correlation between the inhibition of [^{3}H]imipramine binding and the inhibition of [^{3}H]serotonin uptake cannot be taken as proof of an association between the two sites, such a possibility is strongly suggested.

The association of the [^{3}H]imipramine binding site with the mechanism of serotonin uptake is further supported by evidence from other recent studies. A detailed regional distribution of [^{3}H]imipramine binding sites in 23 areas of the rat brain demonstrates a parallelism with the distribution of endogenous levels of serotonin (*12*). This parallelism is even more marked when only the serotonin located in nerve terminals is considered (*12*). Preliminary studies also indicate that electrolytic lesions of

the dorsal raphe nucleus in the rat (a lesion that results in a destruction of serotonin nerve terminals in the hypothalamus) reduce the amount of [^{3}H]imipramine binding in the hypothalamus (*13*).

Finally the existence of [^{3}H]imipramine binding sites in platelets (*4*), a tissue proposed as a model for central monoamine neurons, especially serotonin neurons (*14*), is again compatible with an association between the [^{3}H]imipramine binding site and the neuronal mechanism for serotonin uptake. Additional knowledge about this association and its relationship with the pathogenesis of depression should help to clarify the biological and pharmacological significance of imipramine binding. Results obtained so far suggest that the binding of [^{3}H]imipramine is a useful biological marker in the study of affective disorders and represents a powerful tool in the investigation of the pathogenesis and pharmacotherapy of depression.

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Blood Lead Concentrations in a Remote Himalayan Population

Abstract. *The lead content in the air at the foothills of the Himalayas in Nepal was found to be negligible. The concentration of lead in the blood of 103 children and adults living in this region was found to average 3.4 micrograms per deciliter, a level substantially lower than that found in industrialized populations.*

Human activities have redistributed lead in the environment, contaminating the biosphere (*1*). Although lead is not a required nutrient and has no biological function, it is found in the tissue and blood of individuals in industrialized countries, giving rise to the concept of a "normal" blood lead (Pb-B) concentration. Too much lead in the body, as reflected by a high Pb-B concentration, is associated with human disease, and adverse biological effects can be seen even when the Pb-B concentration is relatively low (*2*). It seems important to determine whether Pb-B concentrations in humans have been increasing as a result of lead contamination of the environment. In 1965, Patterson (*3*) calculated that the Pb-B concentrations of humans before the era of lead pollution was ~ 0.2 μg/dl, or about 100 times lower than the U.S. normal range of 15 to 25 μg/dl. Consistent with this calculation is the finding that the lead content in the bones of ancient Peruvians and Nubians is several times smaller than in the bones of contemporary humans (*4*). Although a few Pb-B measurements obtained before 1970 for some remote populations were reported to be in the normal range (*5*), in 1974 Hecker *et al.* (*6*) reported an average Pb-B concentration of 0.83 μg/dl in the unacculturated Yanomama Indians.

The purpose of our study was to measure the lead content of the air in a remote area and the Pb-B concentration and related hematological parameters in a nonindustrialized but acculturated population living in that area. Two of us (L.C. and M.B.C.), together with four other Americans, took part in an expedition ascending the Marsyandi River in the Manang district of Nepal in the foothills of Annapurna and Dhaulagiri. The route followed trails passable only by foot. The expedition covered 150 miles in 18 days over terrain ranging in altitude from 1400 to 5000 m. The region is devoid of paved roads and industrial development and is populated by people of Tibetan descent. There is no mining and all water is derived from glacial runoff originating above 6000 m.

The expedition required that weight be limited to a minimum, necessitating the use of appropriate microtechniques. Air samples were collected with portable pumps, fitted with 47-mm-diameter Millipore filters, at the rate of 9000 ml/hour; the respirators were run for 12 to 264 hours, collecting between 0.1 and 2.4 m^3 of air (*7*). Lead analyses were performed in the Chapel Hill laboratory of P.M. by flameless atomic absorption spectrophotometry (AAS) (*8*). Simultaneously, 32 other filters, which had been connected to the same respirators at various locations in New York and Virginia for periods of 24 to 144 hours, were analyzed. The control concentrations ranged from 69 ng/m^3 in a centrally air-conditioned laboratory to 1638 ng/m^3 near a first-floor windowsill on a side street in Manhattan—values comparable to those reported by others (*2*). The total lead content of all 12 filters used in Nepal did not differ significantly from the blank, indicating that the lead content of air in the area of Nepal examined is below the measurement technique's limit of detection (10 ng per filter, or 4 ng/m^3).

During the expedition, L.C., after asking consent through an interpreter, obtained blood samples from the local inhabitants by puncturing their fingers and collecting five to ten blood spots, each 1 cm in diameter, on filter paper disks. The disks were allowed to dry in petri dishes for 3 to 5 hours and then were sealed in polyethylene envelopes. Samples were taken from 107 individuals (47 children aged 3 to 12, 30 adult males, and 30 adult females). Four of the six American members of the expedition were sampled at the beginning and at the end of the expedition and were retested by venipuncture 5 weeks later; two were tested only once. These samples provided an internal control, since a very low Pb-B concentration in any of the expedition members would cast doubt on the results for the native population. After the expedition, 103 samples were analyzed blindly for Pb-B at Chapel Hill and 107 samples were analyzed for hemoglobin, erythrocyte protoporphyrin (*9*), and serum ferritin (*10*) in the New York laboratory of S.P.

To validate the measurements of lead in the blood collected on filter paper, samples were collected from 15 normal volunteers in New York by simultaneous venous and finger puncture. The venous blood samples were tested blindly in New York by AAS (*11*) and at Environmental Science Associates by anodic

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