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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stripping voltammetry (*12*). Researchers at Chapel Hill, using the Delves cup method of AAS, blindly tested the venous samples and the samples collected on filter paper (independently coded) and also analyzed 10-μl samples of the venous blood dabbed onto the same type of filter paper (*13*). In New York, the average venous Pb-B concentration was 15.2 μg/dl; in Chapel Hill it was 15.0 μg/dl, 14.6 μg/dl for the samples collected on filter disks, and 14.8 μg/dl for the 10-μl spots, showing excellent agreement between the two laboratories as well as between the samples collected on filter paper and the venous blood samples. The average venous Pb-B concentration measured at Environmental Science Associates was 13.5 μg/dl ($.05 > P > .025$, paired *t*-test with each of the other Pb-B averages). Any bias in the analyses would thus lead to higher results since the samples from Nepal were tested at Chapel Hill.

The Pb-B concentrations in the four Americans ranged between 11.5 and 16.1 μg/dl (average, 14.7 μg/dl) at the beginning of the expedition and between 6.8 and 17.4 μg/dl (average, 10 μg/dl) at the end of the expedition. Five weeks later the concentrations ranged between 10.7 and 16.6 μg/dl (average, 13.3 μg/dl). The Pb-B measurements in the other two members of the expedition were 8.4 and 18.5 μg/dl (*14*).

Figure 1 summarizes the results of all the blood tests on the Himalayan population. The geometric average Pb-B concentration was 3.4 μg/dl (3.8 μg/dl in adult males, 2.9 μg/dl in adult females, and 3.5 μg/dl in children); only 10 of 103 individuals had Pb-B in excess of 10 μg/dl (*15*). These values are the lowest reported for any population except the Yanomama Indians (*6*). For comparison, in a survey of 2515 children in New York it was found that only three had Pb-B below 4 μg/dl; 2405 had concentrations in excess of 10 μg/dl (*16*).

Hemoglobin in the Himalayans averaged 15.4 g/dl (16.1 g/dl in adult males, 15.1 g/dl in adult females, and 15.1 g/dl in children) (*17*). These values are higher than those reported for most Western populations, but are close to those reported for populations living at high altitudes (*18*).

The geometric average erythrocyte protoporphyrin level was 2.2 μg per gram of hemoglobin (1.9 μg/g in adult males, 2.4 μg/g in adult females, and 2.4 μg/g in children) (*19*). These concentrations are of the same order of magnitude as those measured in Western populations and confirm the slightly higher concentrations found in children and females, which probably reflect lower iron stores (*20*).

Measurements of ferritin were obtained for whole blood collected on filter paper (*21*). The geometric average ferritin concentration was 24.2 ng/ml (35.8 ng/ml in adult males, 19.1 ng/ml in adult females, and 21.3 ng/ml in children) (*22*). These results are consistent with findings for similar Western populations, in which a low ferritin concentration is more frequently observed in children and females (*23*).

The range of Pb-B concentrations in the Himalayans is clearly lower than that seen in industrialized peoples. The amounts were slightly higher than those found in the Yanomama Indians and those predicted by Patterson. This could reflect the difference between the sampling techniques or possible exposure of the Himalayans to some natural source of lead (*24*).

In any event, the finding that in two remote populations, one acculturated and one not, the average Pb-B concentration even today is substantially lower than that in industrialized peoples makes it untenable that 15 to 25 μg/dl represents a natural Pb-B level. The higher Pb-B concentrations of people in industrialized countries reflect widespread environmental pollution by this heavy metal, which exacts a toll on essential biochemical systems even at the exposure levels casually accepted as normal (*25*).

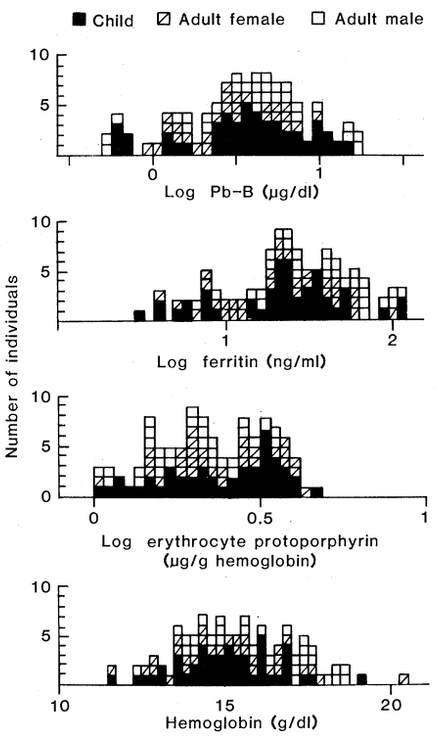

Fig. 1. Distribution of Pb-B, ferritin, erythrocyte protoporphyrin, and hemoglobin in the Himalayans. The population comprises 47 children 3 to 12 years old (46 were used for lead analysis); 30 adult females (27 were used for lead analysis); and 30 adult males. The distributions for Pb-B, ferritin, and erythrocyte protoporphyrin are expressed on a logarithmic scale; the data are grouped with histogram cell widths of 0.06, 0.06, and 0.03 $\log_{10}$, respectively. The distribution of hemoglobin is expressed on an arithmetic scale with a histogram cell width of 0.25 g.

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7. Four portable respirators (DuPont P-200) were calibrated daily. Air samples were collected in Nepal with eight filters of 0.8-μm pore size and four filters of 0.45-μm pore size. These were enclosed in plastic housing and capped except when connected to the air intake port.
8. The lower limit of sensitivity of the measuring system is 10 ng per 5 ml. The lead content of 19 unexposed filters was 370 ng (19 ng per filter); in the 12 filters used in Nepal it was 260 ng (22 ng per filter). When the filters used in Nepal were corrected for background lead, an average difference of 1 ng per filter (7 ng/m^3) was found, a value within the range of variability.
9. Hemoglobin and erythrocyte protoporphyrin

were measured by the method of S. Piomelli [*Clin. Chim. Acta* **23**, 264 (1977)] after the sample was eluted into 0.3 ml of a solution containing 0.1 percent EDTA, 1 m*M* KCN, and 0.06 percent Sterox. The filter paper contained negligible amounts of lead and was precalibrated for sampling homogeneity (10.1 ± 0.5 μl of blood per quarter-inch disk).
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14. The results for the four Americans tested repeatedly suggest a decline in Pb-B by the end of the expedition followed by a return to the previous concentrations after 5 weeks, but are not statistically significant due to the small size of the sample.
15. The $\log_{10}$ average Pb-B concentration (micrograms per deciliter) was 0.531 ± 0.372 (N = 103); for 46 children, 0.544 ± 0.385; for 30 adult males, 0.580 ± 0.411; and for 27 adult females, 0.462 ± 0.278.
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17. The average hemoglobin concentration (grams per deciliter) was 15.4 ± 1.7 (N = 107); for 47 children, 15.1 ± 1.4; for 30 adult males, 16.1 ± 1.7; and for 30 adult females, 15.1 ± 1.9.
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19. The $\log_{10}$ average erythrocyte protoporphyrin concentration (micrograms per gram of hemoglobin) was 0.342 ± 0.189 (N = 103); for 47 children, 0.380 ± 0.188; for 30 adult males, 0.279 ± 0.184; and for 30 adult females, 0.380 ± 0.164.
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21. Ferritin measurements from the heparinized blood samples of 15 controls were obtained for the plasma and for washed red cells and whole blood (both lysed with saponin). From the plasma and red cell ferritins, an expected value for whole blood ferritin was computed and compared to the results obtained for the whole blood and for samples from the same donor which were dabbed onto filter paper. No significant difference was found between the expected values and either set of actual values (paired t-test). The whole blood ferritin concentration did not differ significantly from the plasma ferritin concentration.
22. The $\log_{10}$ average ferritin concentration (nanograms per milliliter) was 1.384 ± 0.359 (N = 107); for 47 children, 1.328 ± 0.360; for 30 adult males, 1.554 ± 0.313; and for 30 adult females, 1.281 ± 0.337.
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Endogenous Substrate for Cyclic AMP–Dependent Protein Kinase in Adrenocortical Polyadenylated Messenger Ribonucleoproteins

Abstract. *An endogenous polysomal cyclic AMP–dependent protein kinase specifically phosphorylates a 150,000-dalton peptide bound to an adrenocortical polyadenylated messenger ribonucleoprotein complex. There is a possibility that this protein is a physiological substrate of cyclic AMP–dependent protein kinase and that the phosphorylation and dephosphorylation of this substrate may be important in the translational control of adrenal polyadenylated messenger RNA.*

Investigations by various laboratories have indicated that protein phosphorylation is an important biological process that mediates several intracellular metabolic activities of eukaryotes (*1*). The phosphorylation reactions are catalyzed by cyclic nucleotide–dependent or cyclic nucleotide–independent protein kinases. Although we have recently provided evidence for the presence of an endogenous polysomal cyclic nucleotide–independent protein kinase that phosphorylates several adrenal proteins associated with polyadenylated messenger RNA [poly(A)mRNA] (*2*), there have been no reports so far of an endogenous protein kinase activated by adenosine 3′,5′-monophosphate (cyclic AMP) that is able to phosphorylate native poly(A)mRNA-associated protein [poly(A)mRNP] particles. Failure to demonstrate this phosphorylation with poly(A)mRNP particles was due to the technical problem of inactivation of protein kinase activity when poly(A)mRNP particles were isolated with conventional techniques of affinity chromatography followed by formamide elution and ethanol precipitation (*2*).

We recently described a technique for isolation of the polysomal particles that

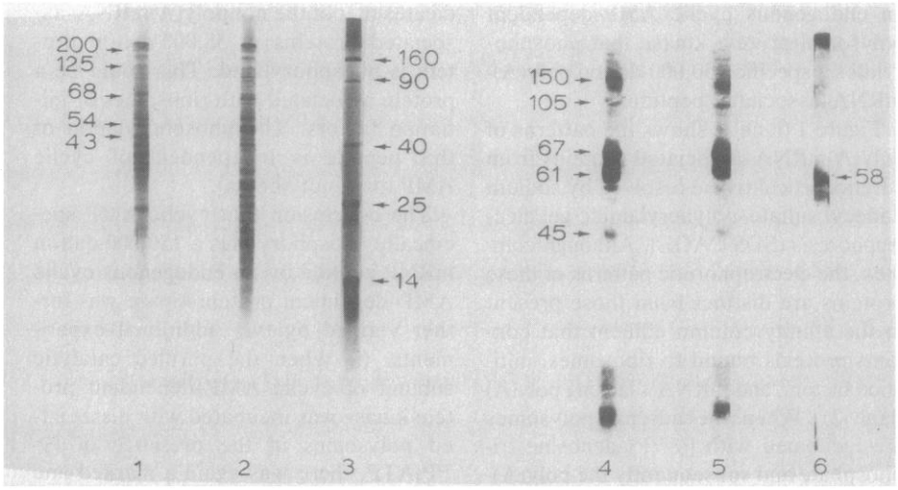

Fig. 1. Characterization of poly(A)mRNA-associated substrates of polysomal cyclic AMP-dependent protein kinases by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Total cytoplasmic polysomes were isolated from bovine adrenal cortex, dissociated in the presence of 1 m*M* puromycin dihydrochloride, and poly(A)mRNP particles were isolated by affinity chromatography on oligo-(dT)-cellulose (*2*). The Coomassie brilliant blue staining pattern of proteins after separation on 12.5 percent polyacrylamide gels (*9*, *10*) is depicted in lanes 1 to 3: (lane 1) poly(A)mRNA-bound proteins, (lane 2) affinity column effluent proteins, (lane 3) molecular weight markers (molecular weight × 1000). Protein kinase activity was assayed by incubation of puromycin-dissociated polysomes with [γ-^{32}P]ATP (28,000 Ci/mmole) prior to separation by affinity chromatography and gel electrophoresis. The ^{32}P incorporated was monitored by autoradiography of dried polyacrylamide gels with Kodak NS-2T x-ray film. Lanes 4 and 5 show the pattern of phosphorylated proteins bound to poly(A)mRNA's after incubation: (lane 4) in the absence and (lane 5) in the presence of the protein kinase inhibitor of cyclic AMP-dependent protein kinase. Lane 6 shows phosphorylated proteins present in the affinity column effluent.

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