conditions the DNA should be half denatured at about 72°C (*13*). Thus, the primary cause of thermal death in *T. acidophilum* could be DNA denaturation, which is not usual with other thermophilic organisms (*14*). Nevertheless, our cultures have remained viable even after having been heated up to 80°C, providing they were then returned to the culture temperature of 59°C. In contrast, at a constant temperature higher than 60°C it is difficult to obtain growth (*1*). Secondly, another natural situation where the protective effect of the histone-like protein could be important is during periods of osmotic shock in fresh water that might occur during a rainstorm or during dispersal to a new environment. *Thermoplasma acidophilum* lacks a cell wall, and, under normal conditions, is in osmotic equilibrium with its environment (*7*). Nevertheless, it can withstand suspension in distilled water and remain viable (*1*), apparently by releasing intracellular potassium ions and other small solutes. Similar mechanisms have been described in other prokaryotes (*15*). Since very low ionic concentrations destabilize DNA (*13*), the histone should also help protect the DNA when the organism is subjected to osmotic shock. This protein can be of significant survival value to *T. acidophilum*, and a similar function might have accounted for the original evolution of eukaryotic histones (*16*).

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β-Endorphin Is Not Detectable in Plasma from Normal Human Subjects

Abstract. *β-Endorphin is not detectable in plasma from normal human subjects when measured under baseline conditions or after the subjects have received vasopressin, an agent that elevates β-lipotropin and adrenocorticotropic hormone (ACTH). Significant amounts of β-endorphin are present in plasma of patients with endocrine disorders associated with increased ACTH and β-lipotropin production. Highly purified, natural β-lipotropin is not peripherally converted to β-endorphin in vivo in normal subjects.*

Adrenocorticotropin (ACTH) and β-lipotropin (β-LPH) are derived from a common precursor glycoprotein molecule (*1*) of approximately 31,000 daltons, and β-LPH may be an obligatory intermediate for the synthesis of β-endorphin (*2*). Immunocytochemical studies have demonstrated the presence of ACTH and β-LPH or β-endorphin (or both) in the same pituitary cells (*3*). The major opioid peptide of rat pituitary extracts is β-endorphin (*4*), and concentrations of this peptide rise in the plasma during acute stress. However, others have reported a higher content of β-LPH than of β-endorphin in rat anterior pituitary (*5*), and we have previously reported that β-LPH is the major opioid (*6*) peptide in human and rat anterior pituitary (*7*).

We now report that β-LPH is the major opioid peptide in normal human plasma. However, β-endorphin is present in high concentration in the plasma of patients with abnormal ratios of ACTH to β-LPH.

We measured β-endorphin (*8*) and β-

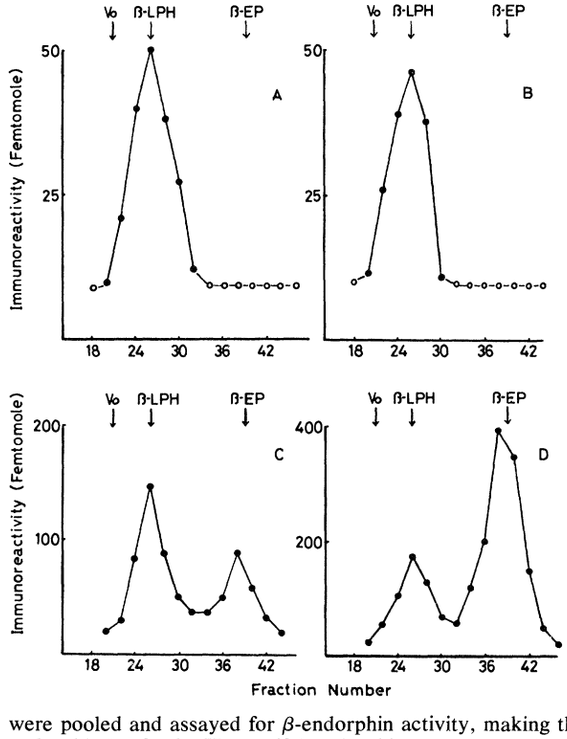

Fig. 1. Sephadex G-50 gel filtration of human plasma extracts from (A) normal subjects at baseline conditions (110-ml pool); (B) normal subjects at time of peak β-LPH levels after vasopressin administration (30 to 60 minutes; 50 ml); (C) a patient with Cushing's disease associated with macroscopic pituitary tumor; and (D) a patient with Nelson's syndrome. Method of quantification of immunoreactivity is described in the legend to Table 1. Arrows indicate the void volume (V_0) (bromophenol blue–bovine serum albumin), and the elution volume peaks of ^{125}I-labeled human β-LPH and ^{125}I-labeled human β-endorphin (*β-EP*). Immunoreactivity is expressed as femtomoles per fraction. The limits of detection for β-endorphin activity in (A) and (B) was 9 fmole per fraction and is indicated by the open circles. Fractions that eluted in the area of the synthetic β-endorphin marker were pooled and assayed for β-endorphin activity, making the effective detection limit for β-endorphin 2.5 fmole. Hence, if β-endorphin were present it would comprise less than 6 percent of the β-LPH activity.

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LPH (*7*, *9*) by radioimmunoassay. Plasma samples were obtained from normal subjects under baseline conditions and after vasopressin stimulation and from patients with Nelson's syndrome, Addison's disease, and Cushing's disease (secondary to macroscopic pituitary tumor). Both peptides were extracted from the plasma (*9*). The recovery of added β-endorphin and β-LPH to 2 ml of plasma was 70 to 80 percent. Extraction efficiency was independent of peptide concentration over the range of 3000 to 12,000 fmole/ml for both peptides. Concentrations less than 3 fmole/ml could also be successfully extracted, albeit at reduced efficiency (52 to 61 percent for 1 fmole/ml). The 31,000 molecular weight precursor, also called pro-opiocortin, has also been quantitatively extracted from human pituitary glands and ectopic ACTH-producing tumors by this technique.

Extracts were subjected to gel filtration on a Sephadex G-50 fine column (1 by 50 cm) and eluted (*10*). Total immunoreactivity of extracts before gel filtration and column immunoreactivity were determined by utilizing antiserum to β-endorphin (Table 1).

After gel filtration, extracts from normal subjects taken under both baseline conditions and after vasopressin exhibited only one peak of immunoreactivity (Fig. 1, A and B), which eluted with ^{125}I-labeled human β-LPH. In all cases the activity in these peaks was equal to or greater than 95 percent of the total immunoreactivity that had been determined on the unchromatographed extracts with antiserum to β-LPH. If β-endorphin activity had been present but comprised less than 6 percent of the total activity on a molar basis, it would not have been detectable on these gel patterns (Table 1 and Figs. 1 and 2). Immunoreactivity in fractions coeluting with ^{125}I-labeled β-endorphin constituted 23 and 34 percent in two patients with Cushing's disease having pituitary tumor, 57 percent in a patient with Addison's disease, and 61 and 71 percent in two patients with Nelson's syndrome (Fig. 1, C and D). No immunoreactive peak was detected in the void volume (corresponding to pro-opiocortin activity) on any of our chromatography patterns.

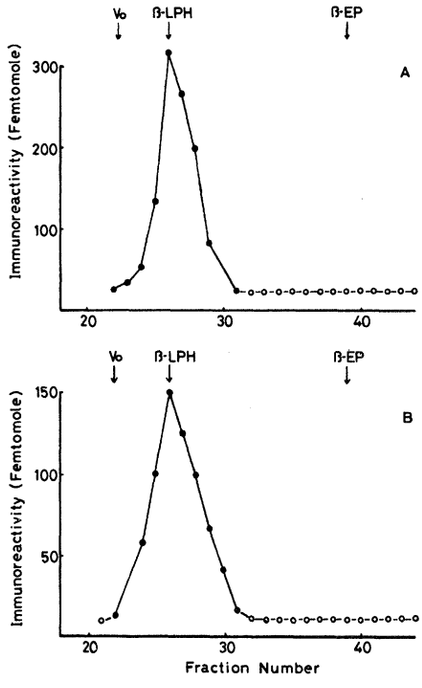

Fig. 2. Sephadex G-50 gel filtration patterns of human plasma extracts (A) 5 minutes or (B) 30 minutes after intravenous bolus injection of human β-LPH. Fractions that eluted in the region of synthetic human β-endorphin (*β-EP*) were pooled before the assay, making the effective detection limit for β-endorphin 2 fmole. If β-endorphin were present in these chromatographs, it would constitute less than 2 percent of the β-LPH activity. When these filtration patterns were assayed with the antiserum to β-LPH, identical peaks (similar K_d and molar activity) were detected (data not shown).

To see whether peripheral conversion of β-LPH to β-endorphin occurred in plasma, we administered 200 μg of highly purified natural human β-LPH intravenously as a bolus to a normal subject. Gel filtration of plasma extracts obtained at 5, 30, 90, and 120 minutes after injection was performed. No measurable conversion of β-LPH to β-endorphin was observed at any time. Filtration patterns of the 5- and 30-minute extracts are shown in Fig. 2. Plasma β-LPH concentrations at 5, 30, 60, 90, and 120 minutes were 12.4, 5.9, 1.0, and 0.7 ng/ml, respectively.

These results agree with our previous report (*7*) that demonstrated the absence of significant β-endorphin activity in normal subjects under baseline conditions and after vasopressin stimulation and from patients with Nelson's syndrome, Addison's disease, and Cushing's disease (secondary to macroscopic pituitary tumor). The 31,000 molecular weight precursor, also called pro-opiocortin, has also been quantitatively extracted from human pituitary glands and ectopic ACTH-producing tumors by this technique.

Table 1. Immunoreactive β-lipotropin (β-LHP) and β-endorphin (β-EP) concentrations. Three separate pools of plasma were obtained from normal subjects under baseline conditions and from each of two studies (with three patients) at the time of peak β-LPH after vasopressin administration. Baseline plasma pools 1 and 2 were each obtained from four normal subjects, while pool 3 was derived from the plasma of a single subject, sampled on two successive mornings between 9 and 11 a.m. Single plasma specimens were obtained in patients with endocrine disease. Total immunoreactive β-LPH was determined on the unchromatographed extracts with a β-LPH antiserum that did not cross-react with β-endorphin but did react with γ-LPH. Immunoreactivity of gel filtration patterns was determined with the antiserum to β-endorphin that reacted on an equimolar basis with β-LPH but not at all with γ-LPH. Molar ratios were obtained by summing the activity in the fractions coeluting with the appropriate labeled reference peptides. This calculation was justified since recoveries of β-LPH and β-endorphin added to the column were similar. In all cases the β-LPH-like activities obtained with antiserum to β-LPH was greater than the values for β-LPH obtained by gel filtration with antiserum to β-endorphin. The percentage difference for baseline values of normal subjects between those values was 12.5, 8.3, and 5.0 percent; after vasopressin administration, they were 26 and 20 percent; for patients with Cushing's disease, the values were 42 and 59 percent; for the patient with Addison's disease, the value was 177 percent; and for the patients with Nelson's syndrome, the values were 242 and 106 percent. The increased activity with the antiserum to β-LPH was probably due to the presence of NH_2-terminal fragments of the β-LPH molecule (for example, γ-LPH) that reacted with the antibody. When the β-LPH and β-endorphin activities are summed, the values obtained closely approximate the total plasma β-LPH activity (β-LPH plus γ-LPH). The available plasma volume in the patients studied was insufficient to further characterize this immunoreactive material.

Clinical condition	Time of sampling	Plasma β-LPH (fmole/ml)	Activity from Sephadex G-50 column (fmole/ml)§		Molar ratio β-LPH/β-EP
			β-LPH	β-EP	
Normal	Baseline				
	Pool 1	4.5	4.0	N.D.†	
	Pool 2	6.3	5.9	N.D.	
	Pool 3	4.2	4.0	N.D.	
Normal	After vasopressin	25.2	20.3	N.D.	
Normal	After vasopressin	38.5	32.1	N.D.	
Cushing's disease	Baseline	185.0	130.3	38.9	3.35
Cushing's disease	Baseline	59.1	37.2	19.2	1.94
Addison's disease	Baseline	225.0	81.2	108.0	0.75
Nelson's syndrome	Baseline	500.0	146.0	356.0	0.41
Nelson's syndrome	Baseline	360.0	138.5	216.0	0.64

*All results are corrected for extraction losses, column recoveries, and volume of plasma extracted and expressed as activity per milliliter of original plasma. †Not detectable. Our limit of detection for β-endorphin was 0.3 to 0.48 fmole/ml.

mal human pituitary, and also agree with the report (*4*) that hyperplastic pituitary fragments from a patient with Nelson's syndrome simultaneously secreted ACTH and β-endorphin when incubated in vitro. Others have suggested (*11*) that β-LPH is released in significant amounts in normal human subjects in response to insulin-induced hypoglycemia. We have reported that plasma ACTH and β-LPH rise in parallel in response to insulin-induced hypoglycemia and vasopressin stimulation in normal subjects (*9*). Our results support the hypothesis (*12*) that ACTH and β-LPH are secreted in the intact form from the pars distalis of normal pituitary. These data also suggest that there is either no significant peripheral conversion in plasma of β-LPH to β-endorphin in normal subjects or that the half-life of plasma β-endorphin is so rapid as to make its presence undetectable. The possibility still remains that peripheral conversion may occur in tissues or at receptor sites.

In patients with pituitary disease (Cushing's disease, Nelson's syndrome) or excessive ACTH production (Addison's disease) who manifest both elevated plasma β-LPH and β-endorphin concentrations, there may be either intrapituitary or peripheral conversion of β-LPH to β-endorphin. Intrapituitary conversion is supported by the study of Guillemin *et al.* (*4*). The half-life of human β-endorphin is longer than that of human β-LPH in the rat (*13*). If similar findings apply to the human, in conditions where increased chronic secretion of β-LPH occurs, the apparent rate of conversion of β-LPH to β-endorphin at unknown sites in these endocrine pathologies would be overestimated, and detectable plasma endorphin concentrations might be expected.

The physiological role of secreted β-LPH in the human is not known, nor is the role of β-endorphin in states of addiction and psychiatric disease (*14*). Whether stresses other than those we have tested can be associated with increased plasma β-endorphin concentrations in the human has not been yet investigated.

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Lecithin Consumption Increases Acetylcholine Concentrations in Rat Brain and Adrenal Gland

Abstract. *Consumption of a single meal containing lecithin, the major source of choline occurring naturally in the diet, increased the concentrations of choline and acetylcholine in rat brain and adrenal gland. Hence, the concentration of acetylcholine in the tissues may normally be under direct, short-term nutritional control.*

We showed previously that the consumption for 3 to 11 days of a diet supplemented with choline chloride (ChCl) sequentially increases the concentrations of serum choline, brain choline, and brain acetylcholine (ACh) in rats (*1*). Such precursor-induced changes in brain (*2*) and adrenomedullary (*3*) ACh concentrations are probably associated with parallel alterations in neurotransmitter release. That similar increases in brain ACh occur after humans ingest choline is suggested by choline's utility in treating tardive dyskinesia, a brain disease thought to be associated with inadequate ACh release (*4*).

Less than 1 percent of the choline normally present in the diet occurs as the free base; most of the remainder is in the form of lecithin (phosphatidylcholine) (*5*). The metabolic fate of choline consumed as lecithin apparently differs from that of free choline: A major fraction of orally ingested choline is rapidly degraded in the human intestine by a bacterial enzyme to yield trimethylamine (*6*), a compound with a marked fishy odor (*7*); in contrast, the consumption of choline as lecithin does not give subjects the fishy odor (*8*) and causes much greater increases in plasma choline, per mole consumed, than does ChCl (*9*). We have examined the effects of choline administered as lecithin, its usual form in the diet, on serum and tissue choline and ACh concentrations in the rat.

Groups of male Sprague-Dawley rats (Charles River) weighing 150 to 250 g were acclimated to our facilities for 1 week prior to experimentation (*10*) and allowed free access to food (Charles River Rat, Mouse, and Hamster Maintenance Formula) and water, except as noted. In experiments on the effects of single meals containing lecithin, rats were fasted overnight, and the following morning they were allowed free access to the experimental diet for 2 hours. They were killed 3, 6, or 10 hours after presentation of the food. Groups of fasting control rats were killed at 0 hour (when the meal began). In the 3-day study, rats consumed all of their daily food intake within a 3-hour period begin-

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