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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ning with the onset of darkness (9:00 p.m.). Care was taken to ensure that all the animals had, in fact, consumed the food provided. For determinations of brain choline and ACh, animals were killed by microwave irradiation of the head (*11*); for determinations of serum choline and adrenal choline and ACh, they were killed by decapitation (*12*). Choline and ACh concentrations were determined radioenzymatically (*13*). Data were analyzed by one-way analysis of variance and Student's *t*-test.

In animals consuming a single meal containing lecithin granules [50 percent by dry weight (*14–16*)], serum choline concentrations increased 62 percent ($P < .01$) within 3 hours, compared with those in animals ingesting neutral fat instead of lecithin, and were still increasing after 10 hours ($P < .001$) (Fig. 1). Choline contents of adrenal glands increased in parallel and were significantly elevated at all times studied. Adrenal ACh concentrations tended to increase throughout the 10-hour period examined, attaining significance (100 percent increase, $P < .01$) after 10 hours. No significant changes in serum or adrenal choline or ACh were observed in fasted rats (0-hour controls) or in animals consuming the 50 percent fat meal (data not shown).

In other single-meal experiments, brain choline and ACh concentrations were examined in rats killed 10 hours after they started to consume a single meal containing lecithin (*14*) (average choline intake was 250 mg/kg). Brain choline increased by 49 percent ($P < .02$) and brain ACh by 19 percent ($P < .01$) (Table 1). The amounts of choline consumed in those short-term studies were within the range that would normally be consumed per day by rats eating our standard laboratory rat chow (*17*).

When animals consuming the lecithin-containing diet (*14*) for 3 days were killed about 12 hours after the last meal, brain choline ($P < .01$) and ACh ($P < .01$) concentrations were still elevated (Table 1). These rats consumed approximately 400 mg of choline per kilogram per day, or about twice the amount that rats might normally be expected to ingest (*17*).

These data indicate that ACh concentrations are significantly increased both in brain (Table 1) and in adrenal glands (where ACh is contained in cholinergic terminals) (Fig. 1) when rats consume within 2 to 3 hours amounts of lecithin not markedly larger than those which they might normally consume within 24 hours. These increases occur after only a doubling in serum choline concentrations (Fig. 1); such increases in serum choline are readily produced in humans by consumption of a meal rich in lecithin (*9*).

High lecithin concentrations occur naturally in such foods as egg yolks (up to 1.75 percent choline), liver (0.6 percent choline), and soybeans (0.3 percent choline) (*5*, *18*); moreover, most processed foods contain lecithin added as an emulsifier (*19*). Reliable data apparently are lacking on the average total daily lecithin intake of adults; however, one estimate is that adults consume 0.5 to 0.9 g of choline per day (*5*, *20*). Normal day-to-day variations in food choice could easily generate tenfold differences in an individual's daily lecithin (and thus choline) intake (*21*). Our data raise the possibility that such variations could influence cholinergic neurotransmission.

Little if any information is available on the possible changes in lecithin metabolism and serum choline concentrations in human diseases (for example, inborn errors of metabolism; gastrointestinal and hepatic diseases). Our data suggest that, if particular diseases do, in fact, alter the metabolism of ingested lecithin, they might also affect cholinergic neurons in the brain and periphery.

The ability of lecithin taken orally to increase brain ACh levels (Table 1) supports our clinical observation that lecithin (*9*), like ChCl (*4*), can be useful in treating tardive dyskinesia (and probably other neurologic disorders characterized by inadequate release of ACh). That the choline in lecithin is not significantly transformed to trimethylamine by gut bacteria probably enhances its clinical effectiveness and certainly improves its acceptability to patients. The lecithin granules used in the present study, like all those currently available commercially in the United States, were only 10 to 20 percent pure; lecithin's clinical utility would probably be enhanced if the pure compound were available inexpensively. Lecithin administration might also provide a useful adjunct to existing drug therapies in diseases (for example, mania, myasthenia gravis, and psychoses) thought to benefit from enhanced cholinergic neurotransmission.

Table 1. Effects of lecithin consumption on brain choline and acetylcholine concentrations. Rats consumed a single meal containing lecithin granules (50 percent by dry weight) for 2 hours (experiment 1) or for 3 days (experiment 2); they were killed 10 hours after access to the single meal (experiment 1) or on the morning of day 4 (experiment 2). Fasting control rats in the 2-hour study were killed at 0 time; control rats in the 3-day study ate a 50 percent neutral fat diet. Data are expressed as means ± standard error.

Group	*N*	Choline (nmole/g)	Acetylcholine (nmole/g)
Experiment 1, single meal			
Control	21	36.5 ± 2.9	21.5 ± 0.8
Lecithin	22	54.3 ± 3.8*	25.6 ± 1.0†
Experiment 2, 3 days			
Control	8	38.5 ± 4.6	21.5 ± 0.8
Lecithin	12	66.1 ± 7.1†	25.6 ± 1.0†

*$P < .02$, differs from controls. †$P < .01$, differs from controls.

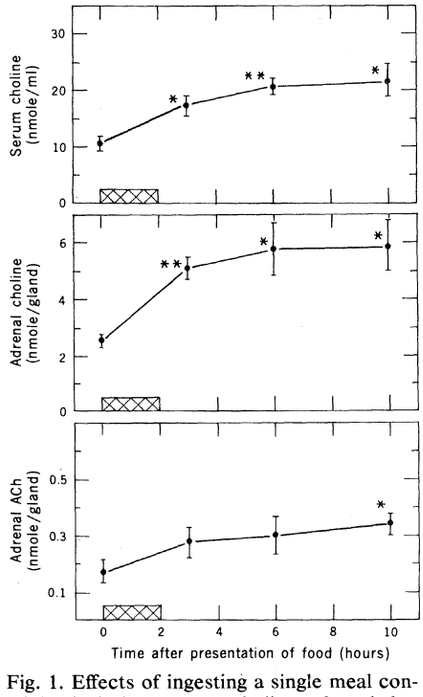

Fig. 1. Effects of ingesting a single meal containing lecithin on serum choline, adrenal choline, and adrenal acetylcholine (ACh) concentrations in rats. After an overnight fast, groups of six to eight animals consumed for 2 hours (hatched bar) a meal containing 50 percent lecithin granules (by dry weight); the food was then removed. Rats consumed an average of 220 mg of choline per kilogram of body weight. Animals were killed at the times indicated. This experiment was repeated two additional times with similar results. Data are expressed as means ± standard error. Asterisk, $P < .01$, compared with controls (0 time); double asterisk, $P < .001$, compared with controls (0 time).

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15. To liberate the choline moiety from the lecithin molecule, lecithin granules (10 mg) were incubated with 10 ml of 1*N* potassium hydroxide for 16 hours at 37°C. This incubation procedure would be expected to liberate choline from glycerophosphorylcholine or other related molecules (for example, lecithin; lysolecithin) containing a phosphorylcholine moiety. Samples of 98 percent pure synthetic dipalmitoyl lecithin (molecular weight 754) (2 or 5 mg) were treated similarly and served as external standards for the survival of the choline and for the completeness of the hydrolysis. Portions of the hydrolysates were assayed for free choline by the radioenzymatic method (*13*). The recovery of the choline from the dipalmitoyl lecithin was 90 percent [E. Baer and M. Kates, *J. Biol. Chem.* **185**, 615 (1950); H. Wittcoff (*16*)].
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21. If an individual were to consume a pound of meat, several egg yolks, a cup of beans, and two doughnuts during the course of a day, his or her choline intake would exceed 5 g.
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Polarity of the Blood-Brain Barrier: Neutral Amino Acid Transport into Isolated Brain Capillaries

Abstract. *Capillary endothelial cells isolated from rat brain exhibit Na^+-dependent uptake of the neutral amino acid analog α-(methylamino)isobutyric acid. Since studies in vivo demonstrate that this transport system is not present on the blood side of brain capillaries we conclude that Na^+-dependent neutral amino acid transport is located on the brain side. Therefore, the luminal plasma membrane and the antiluminal plasma membrane appear to be functionally distinct. This polarity should permit brain capillary endothelial cells to actively regulate the internal milieu of the brain.*

Numerous studies in vivo show that some polar solutes easily enter the brain from the blood, while others do not. This selective permeability barrier between the blood and brain is called the blood-brain barrier (BBB). Thus, methods which measure unidirectional uptake of amino acids from the blood readily demonstrate a BBB transport system for large neutral amino acids such as phenylalanine, leucine, tryptophan, and methionine (*1–4*); however, there is little or no transport of small neutral amino acids such as glycine, alanine, serine, and proline (*1, 3, 4*). These two groups of amino acids correspond respectively to the Na^+-independent L system and the Na^+-dependent A system for neutral amino acid transport into other cells (*5*). Therefore, it has been concluded that the A system for amino acid transport does not exist in the BBB (*3, 4, 6*). The observation that the A-system analog, α-aminoisobutyric acid, does not enter brain during a single capillary passage supports this hypothesis (*1, 7*). Furthermore, after intravenous administration this amino acid accumulates in most tissues of the body, but not in the brain (*8*). Despite the apparent absence of Na^+-dependent (concentrative) amino acid transport in the BBB, some A-system amino acids can be transported from brain to blood against a concentration gradient (*9*). These results raise the possibility that the brain side of the BBB differs from the blood side. However, interpretation of these studies in vivo is complicated by the presence of amino acid transport systems in choroid plexus (*10*) and brain cells (*11*).

All evidence indicates that the plasma membrane and intercellular tight junctions of the brain capillary endothelial cells are responsible for the BBB (*12*). The development of methods for isolating capillaries from brain has made it possible to investigate the transport properties of the BBB directly at a cellular level (*13*). Thus, Sershen and Lajtha have shown that the L system for amino acid transport is present in isolated brain capillaries and that it is Na^+-independent (*4*). However, these investigators did not study in vitro the capillary uptake of an A-system analog. We now report on the uptake of α-(methylamino)isobutyric acid (αMeAIB) and L-leucine by isolated brain capillaries. We chose these amino acids because in other cells, αMeAIB is transported exclusively by the Na^+-dependent A system while leucine is transported principally by the Na^+-independent L system (*5*).

Capillaries were isolated from the cerebral cortex of rats that were 30 days old by a method that has been described (*13*). Amino acid uptake by isolated capillaries was followed in the presence or absence of Na^+ or with the Na^+ gradient abolished by ouabain. Figure 1A shows that αMeAIB is concentrated within the endothelial cells in the presence of Na^+ and that this accumulation is prevented by prior incubation with ouabain. Leucine also exhibits a minor Na^+-depen-

Table 1. Stereospecificity of the A system (αMeAIB) and L system (L-leucine) for neutral amino acid transport into isolated brain capillaries. The uptake of αMeAIB was measured after incubation for 10 minutes in the presence of 150 m*M* NaCl buffer and 0.3 m*M* ^{14}C-labeled αMeAIB (2.5 μCi/ml), with or without 10 m*M* inhibiting amino acid. The uptake of L-leucine was measured after incubation for 1 minute in the presence of 150 m*M* choline chloride buffer and 0.15 m*M* L-[1-^{14}C]leucine, with or without 10 m*M* inhibiting amino acid. Symbols: ++++, 50 to 60 percent inhibition; +++, 40 to 50 percent inhibition; ++, 30 to 40 percent inhibition; +, 10 to 30 percent inhibition; and 0, < 5 percent inhibition. Results are the averages of three determinations. All standard deviations are less than 7 percent inhibition.

Inhibiting amino acid (10 m*M*)	Inhibition of αMeAIB uptake	Inhibition of leucine uptake
αMeAIB	++++	0
L-Proline	++++	0
L-Methionine	++++	++++
L-Leucine	+++	++++
L-Phenylalanine	+++	++++
L-Alanine	+++	+++
Glycine	++	+
L-Tryptophan	++	++++
L-Serine	+	+++
L-Histidine	+	+++

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