4. K. L. Davis, L. E. Hollister, J. D. Barchas, P. A. Berger, *Life Sci.* **19**, 1507 (1976); J. H. Growdon, M. J. Hirsch, R. J. Wurtman, W. Weiner, *N. Engl. J. Med.* **297**, 524 (1977).
5. C. H. Best and C. C. Lucas, in *Clinical Nutrition*, N. Jolliffe, Ed. (Harper, New York, 1962), pp. 227–260.
6. J. De La Huerga and H. Popper, *J. Clin. Invest.* **30**, 463 (1951).
7. C. W. Lee, J. S. Yu, B. B. Turner, K. E. Murray, *N. Engl. J. Med.* **295**, 937 (1976).
8. J. H. Growdon, A. J. Gellenberg, J. Doller, M. J. Hirsch, R. J. Wurtman, *ibid.*, **298**, 1029 (1978).
9. R. J. Wurtman, M. J. Hirsch, J. H. Growdon, *Lancet* **1977-II**, 68 (1977); M. J. Hirsch, J. H. Growdon, R. J. Wurtman, *Metabolism*, **27**, 953 (1978).
10. Animals were exposed to light (Vita-Lite, 300 μW/cm^2; Duro-Test Corp.) for 12 hours per day.
11. A focused microwave beam from modified Litton microwave oven (Medical Engineering Consultants) was directed to the brain for 3.5 seconds; this prevents postmortem hydrolysis of brain ACh [W. B. Stavinoha, S. T. Weintraub, A. T. Modak, *J. Neurochem.* **23**, 885 (1973)].
12. Adrenal glands were quickly removed and frozen on Dry Ice; blood was collected on ice from the cervical wound and centrifuged at 4°C to separate the serum.
13. P. A. Shea and M. H. Aprison, *Anal. Biochem.* **56**, 165 (1973); E. L. Cohen and R. J. Wurtman, *Life Sci.* **16**, 1095 (1975).
14. Lecithin granules (Sigma) contained 10 to 20 percent lecithin and 80 to 90 percent mixed neutral lipid; the total choline content of each batch was analyzed (*15*), and the average choline intake of each rat calculated (220 mg/kg). For the single-meal studies, the experimental diet contained, per 1000 g dry weight, sucrose (128 g), dextrose (138 g), dextrin (138 g), Rogers-Harper Mineral Mix (40 g), vitamin mix minus choline (22 g; Nutritional Biochemical Corp.), agar (34 g), either lecithin granules or corn oil (Mazola) (500 g), and water (1000 g). For the 3-day study, the diet contained vitamin-free casein (100 g), sucrose (100 g), dextrose (102 g), dextrin (102 g), vitamin mix minus choline (22 g), Rogers-Harper Mineral Mix (40 g), agar (34 g), lecithin granules or corn oil (Mazola) (500 g), plus water (1000 g).
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*)].
16. H. Wittcoff, *The Phosphatides* (Reinhold, New York, 1951).
17. Charles River Rat, Mouse, and Hamster Maintenance Formula contains 0.18 percent choline. A normal rat would be expected to consume about 10 to 15 percent of its body weight in food daily; this would supply 180 to 250 mg of choline per kilogram per day.
18. R. S. Goodhart and M. E. Shils, Ed., *Modern Nutrition in Health and Disease* (Lea & Febiger, Philadelphia, ed. 5, 1975), p. 261; W. H. Griffith and J. F. Nyc, in *The Vitamins*, W. H. Sebrell and R. S. Harris, Eds. (Academic Press, New York, ed. 2, 1971), vol. 3, pp. 76–80.
19. Lecithin is routinely added to chocolate (0.3 percent lecithin), margarine (0.1 to 0.5 percent lecithin), ice cream (0.5 percent lecithin), and bakery goods (up to 2 percent lecithin in bread dough and cake batter) (*16*).
20. L. J. Bogert, G. M. Briggs, D. H. Calloway, *Nutrition and Physical Fitness* (Saunders, Philadelphia, 1973), p. 153.
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.
22. These studies were supported by grants from the Ford Foundation, the National Institute of Mental Health (MH-28783), and the National Aeronautics and Space Administration (NGR-22-009-627). M.J.H. holds an NIMH predoctoral fellowship (MH-05479-01).

17 April 1978; revised 30 May 1978

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	+	+++

0036-8075/78/1013-0225$00.50/0

dent accumulation. In other cells the A system can be selectively eliminated by incubation in the absence of Na^+ (*14*). Figure 1B shows that αMeAIB uptake by brain capillaries is markedly decreased in the absence of Na^+. By contrast, leucine shows rapid uptake even when Na^+ is eliminated and this process is not inhibited by αMeAIB. Thus, in brain capillaries the A and L systems for neutral amino acid transport can be distinguished by incubation in the presence or absence of Na^+. The stereospecificity for αMeAIB uptake in the presence of Na^+ (A system) and for leucine uptake in the absence of Na^+ (L system) is shown in Table 1. The results demonstrate some overlap in affinities for the two transport systems; however, this pattern is nearly identical to that obtained with Ehrlich ascites cells in which the A and L systems were first described in detail (*5*).

Our results clearly demonstrate the existence of Na^+-dependent (A system) and Na^+-independent (L system) neutral amino acid transport into isolated brain capillaries (*15*). In this preparation in vitro, the incubation medium has access to both the luminal plasma membrane, which is normally in contact with the blood, and the antiluminal plasma membrane, which is normally in contact with the brain interstitial fluid. Since the results of numerous studies in vivo indicate that A-system transport does not occur from the luminal side of the brain capillary (*1, 3, 4, 7, 8*), we conclude that this transport system is located on the antiluminal side. This polarity for A-system amino acid transport would explain the active efflux of small amino acids from brain to blood despite their limited entry from blood to brain (*1, 3, 4, 9*). In contrast, the L system is probably located on both the luminal and antiluminal sides.

Other solutes such as K^+, iodide, prostaglandins, and certain organic acids which are not readily taken up from the blood exit from the brain against a concentration gradient (*16*). Thus, these transport systems may also have a polar distribution in the endothelial cell membrane. The operation of such antiluminal active transport systems, whether directly coupled to energy or coupled indirectly through the sodium gradient, would require a substantial energy input. This may explain the relatively high mitochondrial density of brain capillary endothelial cells (*17*).

Epithelial cells which line the lumen of the small intestine and the proximal tubule of the kidney are also polar (*18*). The luminal membranes of these cells contain Na^+-dependent glucose and amino acid transport systems and the enzyme alkaline phosphatase, whereas Na^+-independent solute transport and Na^+- and K^+-dependent adenosinetriphosphatase are located in the antiluminal membrane. Besides polarity, other similarities with brain capillary endothelial cells include the presence of intercellular tight junctions (*19*), a high activity of alkaline phosphatase (*20*) and γ-glutamyltranspeptidase (*21*), and the presence of a large number of mitochondria (*17*). Thus, in many respects, cerebral endothelial cells are similar to polar epithelial cells in other tissues.

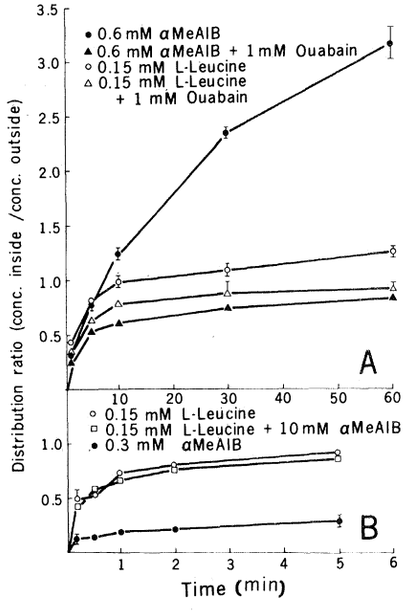

Fig. 1. The uptake of ^{14}C-labeled αMeAIB or L-[1-^{14}C]leucine was measured at 37°C in a total incubation volume of 0.2 ml containing 0.1 to 0.2 mg of capillary protein, 0.5 to 1.0 μCi of ^{14}C, and the stated concentration of unlabeled amino acid. The incubation buffer consisted of 150 m*M* NaCl (A) or 150 m*M* choline chloride (B) and 4 m*M* KCl, 3.2 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, 15 m*M* Hepes (*p*H 7.4), 5 m*M* glucose, and 1 percent (weight to volume) bovine serum albumin. The reaction was stopped by filtration through a glass fiber filter and the cells were washed with ice-cold Hepes-buffered Ringer solution. The washed filters were immediately placed in 6 percent (weight to volume) perchloric acid and sonicated for 5 minutes. After centrifugation, the supernatant was counted in a liquid scintillation spectrometer. For the blank, a portion of cells was added to the ice-cold Hepes-buffered Ringer solution before the addition of isotope. Calculation of the distribution ratio was based on an intracellular water content of 4.2 μl per milligram of protein. This value was determined previously (*22*) as the difference between the wet and dry weight of the preparation minus the L-glucose space. Results are the average of three determinations; standard deviations not shown were less than the size of the symbol. (A) Cells were first incubated for 20 minutes at 37°C with or without 1 m*M* ouabain. (B) Cells were prepared in NaCl buffer but then washed three times with a buffer containing an equimolar concentration of choline chloride and no NaCl. After 20 minutes of incubation at 37°C, uptake experiments were performed in the choline buffer.

In conclusion, we propose that the endothelial cells in brain capillaries have a polar distribution of solute transport carriers. Limitation of the Na^+-dependent amino acid transport system to the antiluminal plasma membrane of these cells allows for the transfer of selected amino acids from brain to blood against a concentration gradient. Similarly, a polar distribution of ion and organic acid transport carriers would permit active regulation of the composition of brain interstitial fluid and the net secretion or absorption of this fluid by brain capillaries.

A. LORRIS BETZ
GARY W. GOLDSTEIN
Departments of Neurology and *Pediatrics, University of California, San Francisco 94143*

References and Notes

1. W. H. Oldendorf, *Am. J. Physiol.* **221**, 1629 (1971).
2. A. L. Betz, D. D. Gilboe, L. R. Drewes, *ibid.* **228**, 895 (1975); W. H. Oldendorf, *ibid.* **224**, 967 (1973).
3. D. L. Yudilevich, N. DeRose, F. V. Sepulveda, *Brain Res.* **44**, 569 (1972).
4. H. Sershen and A. Lajtha, *Exp. Neurol.* **53**, 465 (1976).
5. H. N. Christensen, *Adv. Enzymol.* **32**, 1 (1969); *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 19 (1973).
6. L. A. Wade and R. Katzman, *J. Neurochem.* **25**, 837 (1975); W. M. Pardridge, *ibid.* **28**, 103 (1977).
7. R. Blasberg, C. A. Patlak, J. W. Jehle, J. D. Fenstermacher, *Neurology* **23**, 363 (1978).
8. R. J. Schain and K. S. Watanabe, *J. Neurochem.* **19**, 2279 (1972).
9. A. Lajtha and J. Toth, *ibid.* **8**, 216 (1961); J. E. Murray and R. W. P. Cutler, *Arch. Neurol.* **23**, 23 (1970); A. V. Lorenzo and S. R. Snodgrass, *J. Neurochem.* **19**, 1287 (1972).
10. A. V. Lorenzo and R. W. P. Cutler, *J. Neurochem.* **16**, 577 (1969); L. A. Coben, E. Cotlier, C. Beaty, B. Becker, *Brain Res.* **30**, 67 (1971).
11. S. R. Cohen and A. Lajtha, in *Handbook of Neurochemistry*, A. Lajtha, Ed. (Plenum, New York, 1972), vol. 7, pp. 543–572.
12. T. S. Reese and M. J. Karnovsky, *J. Cell Biol.* **34**, 207 (1967); M. W. Brightman and T. S. Reese, *ibid.* **40**, 648 (1969); T. S. Reese, M. Feder, M. W. Brightman, *J. Neuropathol. Exp. Neurol.* **30**, 137 (1971); S. I. Rapoport, *Blood-Brain Barrier in Physiology and Medicine* (Raven, New York, 1976), pp. 63–74.
13. G. W. Goldstein, J. S. Wolinsky, J. Csejtey, I. Diamond, *J. Neurochem.* **25**, 715 (1975).
14. Y. Inui and H. N. Christensen, *J. Gen. Physiol.* **50**, 203 (1966).
15. Although the capillary preparation consists predominantly of endothelial cells, there is some contamination by erythrocytes and pericytes (estimated at less than 5 and 10 percent, respectively). It is unlikely that erythrocytes contribute to the observed accumulation of αMeAIB because the A system is not present in these cells (*5*). In the absence of an Na^+ gradient, αMeAIB equilibrated with the water space of the entire preparation (Fig. 1A), that is, endothelial cells and pericytes combined. Thus, A-system transport by the pericytes could not by itself account for our results. Because the choroid plexus is removed before the cortex is homogenized, these cells are not a contaminant in the final preparation.
16. H. Davson, *J. Physiol. (London)* **225**, 1 (1976).
17. W. H. Oldendorf, M. E. Cornford, W. J. Brown, *Ann. Neurol.* **1**, 409 (1977).
18. H. Murer and U. Hopfer, in *Intestinal Permeation*, F. Lauterbach and M. Kramer, Eds. (Excerpta Medica, Amsterdam, 1976), pp. 294–312; R. Kinne, H. Murer, E. Kinne-Saffran, M. Thees, G. Sachs, *J. Membrane Biol.* **21**, 375 (1975).

19. M. G. Farquhar and G. E. Palade, *J. Cell Biol.* **17**, 375 (1963); A. R. Muir and A. Peters, *ibid.* **12**, 443 (1962).
20. S. L. Manocha, *Histochemie* **21**, 221 (1970).
21. Z. Albert, M. Orlowski, A. Szewczuk, *Nature (London)* **191**, 767 (1961); M. Orlowski, G. Sessa, J. P. Green, *Science* **184**, 66 (1974).
22. A. L. Betz, J. Csejtey, G. W. Goldstein, *Am. J. Physiol.*, in press.

23. We thank J. Csejtey and A. Ste. Marie for technical assistance. Supported by NIH grants ES 01164 and HL 22088 and by the National Foundation–March of Dimes. A.L.B. is the recipient of the NIH postdoctoral fellowship NS 05807. G.W.G. is the recipient of the NIH RCDA NS 00278.

18 April 1978; revised 24 May 1978

Cocaine Plasma Concentration: Relation to Physiological and Subjective Effects in Humans

Abstract. *Volunteer subjects with previous histories of cocaine use were administered cocaine hydrochloride intravenously or intranasally. There was a positive relationship between peak plasma concentration, physiological and subjective responses, and dose administered. The rate of cocaine disappearance after intravenous administration paralleled the drop in physiological and subjective drug effects. After intranasal administration, blood levels remained elevated for a considerably longer period.*

The presence of a positive correlation between plasma levels of cocaine in humans and its physiological and subjective effects has been questioned in two recent reports (*1*, *2*). Van Dyck *et al.* (*1*) applied a 10 percent solution of cocaine hydrochloride to the nasal mucosa of surgical patients (who were also given other drugs), and found that the peak concentrations in plasma occurred after approximately 60 minutes. They noted that since maximum euphoria reported by street users occurs within 3 to 5 minutes after inhalation of the drug, the peak plasma levels of cocaine are not related to peak euphoric effects. Resnick *et al.* (*2*) administered cocaine solutions intravenously and intranasally and found that peak drug effects occurred within 5 to 10 minutes after intravenous injection and 15 to 20 minutes after intranasal administration, but did not measure plasma levels. Using for comparison the data of Van Dyck *et al.* (*1*), who measured plasma levels but not subjective effects, Resnick *et al.* concluded that the time course of the physiological and subjective effects is apparently not related to levels of cocaine in plasma.

The present study was designed to correlate physiological and subjective effects with the plasma concentration of cocaine in the same subjects (*3*). Ten adult volunteers with histories of intravenous cocaine use were the subjects. Prior to inclusion in the study, each was given an extensive drug history interview and a thorough psychiatric and physical examination (*4*). Subjects were admitted to the clinical research unit of Billings Hospital for a 2-week period during which they were tested daily with either intravenous cocaine or saline or intranasal cocaine. Cocaine hydrochloride (16 or 32 mg) dissolved in physiological saline was administered intravenously (1 ml in 60 sec) through a previously inserted scalp-vein infusion set. Intranasal cocaine was administered as 100 mg of white powder consisting of the appropriate dose of cocaine (16, 64, and 96 mg) mixed with lactose powder. Subjects were instructed to inhale this mixture through a 5.0-cm straw within 1 minute.

One or two scalp-vein butterfly infusion sets attached to saline drip bags were inserted into arm veins and appropriate physiological monitoring devices were placed on each subject 1.5 hours before drug administration. Heart rate was continuously recorded, and subjects were required to report on their drug "high" and fill out drug effects questionnaires, which included identifying the drug they were given and rating it on a 0 to 10 scale (0 = placebo, 5 = average dose of "street" cocaine, 10 = largest dose of "street" cocaine ever taken) (*5*).

Blood samples were collected through a scalp-vein infusion set. When the route of drug administration was intravenous, blood was withdrawn from the arm opposite to the infusion arm. Sodium fluoride (2.5 mg per milliliter of blood) was added to each sample, which was then mixed and separated into plasma and red blood cells. Cocaine from the sample was extracted without delay and determined by gas chromatographic method using an electron capture detector (*6*). Samples were identified by code only, and the code was not broken until all subjective and physiological data had been analyzed.

Table 1. Plasma concentrations of cocaine after intravenous administration. Cocaine values are given as mean ± standard error; *n*, number of determinations.

Time since drug (min)	Dose			
	16 mg		32 mg	
	Cocaine (ng/ml)	*n*	Cocaine (ng/ml)	*n*
5	221 ± 41	12	308 ± 32	15
10	164 ± 24	14	253 ± 25	19
20	150 ± 22	9	253 ± 45	8
30	109 ± 13	14	170 ± 19	19
60	62 ± 9	14	111 ± 14	20
90	30 ± 3	5	61 ± 10	14
120	21 ± 4	5	49 ± 10	10

Plasma concentrations of cocaine after intravenous injections of 16 and 32 mg are given in Table 1. Plasma levels were higher after the larger dose. There were large intersubject variations in the plasma concentrations in the ten subjects studied (for instance, cocaine plasma concentrations ranged from 86 to 309 ng/ml 5 minutes after a 16-mg injection and from 216 to 409 ng/ml 5 minutes after a 32-mg injection). Half-life of cocaine disappearance from plasma varied from 16 to 87 minutes (*7*). This is the first study of plasma half-life of cocaine in humans after intravenous injection, although Misra (*8*) reported plasma half-life of cocaine after intravenous injection in rats, dogs, and monkeys of 18, 72, and 72 to 78 minutes, respectively.

The rate of cocaine disappearance in human plasma after intravenous injection paralleled the return of heart rate to predrug levels after the peak increase 8 to 12 minutes after cocaine administration (Fig. 1). Although some intersubject variability was seen, changes in heart rate after intravenous cocaine were similar to those previously reported (*4*), with heart rate returning to predrug levels by 30 to 45 minutes. After intravenous injection of drug, subjects reported that the maximum "high" occurred approximately 3 to 5 minutes after injection. They indicated that the drug effect had disappeared within 30 to 40 minutes and that, if self-injecting, they would be ready for a second dose at this time. Both plasma concentration of cocaine and physiological and subjective effects were dose-related. It appears that the cardiovascular and subjective effects are highly correlated with the rapid increase in cocaine plasma levels, peaking early and showing parallel decreases over the first 30 minutes after intravenous injection.

Plasma concentrations of cocaine after different intranasal doses are given in Table 2. Concentrations increased rapidly for the first 20 to 30 minutes after inhalation, reaching peak levels before 60 minutes, and then decreased gradually over the next hour. Higher doses yielded higher peak plasma values. As was ob-

0036-8075/78/1013-0227$00.50/0