a highly fluorescent DNA-DAPI complex (*3*). This binding appears to be dependent upon nucleotide content, selectively binding to adenine-thymine–rich DNA. DAPI is highly sensitive and has been used as a cytochemical means for detecting mycoplasma contamination in tissue culture systems (*4*).

Three strains of *T. equiperdum*, the normal kinetoplastic strain (ATCC 30019), the spontaneously dyskinetoplastic strain (ATCC 30023), and an acriflavin-induced dyskinetoplastic strain, were used in this study. The trypanosomes were grown in albino mice, and tail blood samples of exponentially growing cells were diluted with an equal volume of phosphate-saline-glucose buffer (*p*H 8) containing DAPI at a concentration of 0.1 μg/ml and incubated at 37°C for 10 to 20 minutes. A small drop of the incubated sample was placed on a slide, covered with a cover glass, pressed to form a very thin film, and examined by fluorescence microscopy.

Fluorescence is restricted to the kinetoplast and nucleus of the kinetoplastic strain of *T. equiperdum*. The nucleus appears as a large blue fluorescent structure in approximately the center of the cell; the kinetoplast appears either as a small blue fluorescent body (Fig. 1a) or as a pair of fluorescent bodies following kinetoplast division (Fig. 1b). Fluorescence in the spontaneously dyskinetoplastic strain of *T. equiperdum* is not limited to the nucleus and kinetoplast regions of the cell (Fig. 1, c and d). Small fluorescent bodies of varying size and intensity occur in the kinetoplast region and throughout the cell, generally arranged as a row of particles (Fig. 1c). This ordered distribution of DNA-DAPI complexes suggests that the DNA is restricted from dispersal in the cytoplasm by the mitochondrial membrane. The nucleus retains the staining characteristics of the normal kinetoplastic cells. The acriflavin-induced dyskinetoplastic cells are ultrastructurally indistinguishable from the spontaneously dyskinetoplastic cells and show identical staining properties with DAPI (Fig. 1, e and f). The evidence presented here confirms the interpretation that the clumps of electron-dense material seen in electron micrographs of dyskinetoplastic cells are DNA and supports the hypothesis of several investigators (*1*, *5*) that the failure of the kinetoplast of dyskinetoplastic trypanosomes to stain with Giemsa and Feulgen stains is due to dispersion of the K-DNA throughout the mitochondrion rather than a reduction in the total amount of K-DNA.

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6. I thank Professor Otto Dann for his generous gift of DAPI, Dr. D. H. Williamson for his personal communication, and Dr. W. B. Cosgrove for advice and encouragement.

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Cocaine: Plasma Concentrations After Intranasal Application in Man

Abstract. *Cocaine (1.5 milligrams per kilogram) was applied to the nasal mucosa of human subjects. The cocaine persisted in the plasma for 4 to 6 hours and reached peak concentrations of 120 to 474 nanograms per milliliter at 15 to 60 minutes. In that residual cocaine was detectable on the nasal mucosa for 3 hours, continuous absorption secondary to its vasoconstrictive action might explain its persistence in the plasma.*

Using gas-liquid chromatography (GLC) with a nitrogen detector, we have measured cocaine plasma concentrations in humans who had been given a therapeutic dosage. To our knowledge, the only previously reported determination in humans was after a fatal overdosage (*1*). Measurement of cocaine concentrations in plasma after administration of clinical or street dosages had to await the development of sensitive and specific analytic methods.

Cocaine is a nonsynthetic local anesthetic and a stimulant of the central nervous system. In animals it is known to block the reuptake of endogenous amines in the sympathetic nervous system and to potentiate the effects of exogenous amines (*2*). As a local anesthetic it has the added advantage of causing vasoconstriction and is widely used in otolaryngology and in anesthesiology. Socially, cocaine is a major drug of abuse that has dramatically increased in popularity. It is reported to produce an intense euphoria 3 to 5 minutes after intranasal application and thereby has gained the reputation of being the "champagne of drugs." Freud (*3*) advocated its use as an antidepressant, and Post *et al.* (*4*) gave it orally (up to 200 mg) and intravenously (up to 25 mg) to five depressed patients who showed no improvement. Long-term administration of cocaine, like amphetamine, is reported to cause a toxic psychosis in man (*5*). Despite its long history and wide usage, little is known about the systemic effects of cocaine in man. As the first part of an effort to elucidate its pharmacology in man, we measured the plasma concentrations of cocaine in 13 surgical patients who were given the drug as a vasoconstrictor prior to nasal intubation.

The subjects were nine patients undergoing cardiovascular surgery (CV patients) (*6*) and four patients undergoing dental surgery (dental patients) (*7*), who were informed about the study and agreed to participate. A 10 percent solution of cocaine hydrochloride (1.5 mg per kilogram of body weight) was applied topically to the nasal mucosa prior to nasal intubation. All patients received diazepam (10 mg per 70 kg of body weight) and morphine sulfate (10 mg per 70 kg of body weight) intramuscularly 1 hour before being given general anesthesia. The CV patients were given diazepam or sodium pentothal and either succinylcholine or pancuronium bromide intravenously for muscle relaxation, while the dental patients were given sodium pentothal and succinylcholine intravenously. Both groups received nitrous oxide and halothane as maintenance anesthetics.

In all patients, we obtained 10-ml blood samples before application of cocaine, and samples were again taken 10, 15, 20, and 60 minutes after application. We obtained additional blood samples at 3 and 6 minutes in eight patients to detect any early peak values, which otherwise might have been missed. No samples were collected from CV patients after they were placed on cardiopulmonary bypass since this increased the volume of blood in the system and would have rendered subsequent plasma concentrations invalid. For this reason we do not have data on plasma concentrations in these patients beyond 3 hours and 40 minutes after cocaine application. We obtained samples from dental patients for as long as 6 hours after drug application. All blood samples were collected in heparinized glass syringes, and were immediately placed in ice and transported to the laboratory where the plasma was separated and analyzed with minimal delay (*8*).

Plasma concentrations of cocaine were determined by a GLC equipped with a nitrogen detector (*9*). Samples were prepared

Table 1. Time course of cocaine hydrochloride concentrations in the plasma of cardiovascular patients. The values are expressed as means ± standard errors. The numbers in parentheses indicate the number of patients.

Time (min)	Cocaine in plasma (ng/ml)
0	3.7 ± 1.4 (9)
10	182.4 ± 37.3 (9)
15	239.8 ± 42.8 (9)
20	256.5 ± 33.4 (9)
60	308.4 ± 33.2 (9)
150	278.0 ± 56.3 (2)
180	205.8 ± 61.2 (4)
220	132.0 ± 36.7 (2)

by adjusting 2 ml of plasma to *p*H 9.6 to 9.8 with carbonate buffer, then extracted with 5 ml of a mixture of heptane and isoamyl alcohol (98 : 2) containing the propyl ester of benzoylecgonine as an internal standard (*10*). Following back extraction with 1 ml of 0.1*N* H_2SO_4, the sample was washed with solvent, alkalinized to *p*H 9.6 to 9.8 with a solid mixture of Na_2CO_3 and $NaHCO_3$, and reextracted into a mixture of heptane and isoamyl alcohol. The solvent was evaporated at room temperature under nitrogen, and a portion of the residue was dissolved in methanol and injected into a gas chromatograph (*11*) equipped with a nitrogen detector. Standards prepared by addition of cocaine to samples of the subject's plasma at zero time were analyzed in an identical manner. The coefficient of variation (standard deviation : mean) of the procedure at a cocaine concentration of 100 ng/ml was 3.0 percent. With this procedure, drug-free plasma showed a baseline which prevented detection of cocaine concentrations less than 2.0 ng/ml.

Plasma concentrations of cocaine increased rapidly for 15 to 20 minutes, peaked at 15 to 60 minutes, and then decreased gradually over the next 3 to 5 hours (Tables 1 and 2). Cocaine was measurable in the blood within 3 minutes and for as long as 6 hours after application. Maximum plasma concentrations ranged from 120 ng/ml to 474 ng/ml calculated as the free base.

The maximum plasma concentrations of CV patients were significantly greater than the maximum plasma concentrations of dental patients ($P = .001$; Mann-Whitney two-sample test). A number of factors could explain this difference (*12*). The CV patients were older [52.1 ± 2.9 years, mean ± standard error (S.E.)] and heavier (76.1 ± 4.9 kg) than the dental patients (33.2 ± 11.4 years, 66.5 ± 2.0 kg). Similarly, Berkowitz *et al.* (*13*) reported that older patients had higher plasma concentrations after the same dosage of morphine sulfate. The CV patients also had abnormal cardiovascular function with lowered cardiac output. In fact, four patients were in congestive heart failure at the time of surgery, and three of these four patients had the three highest peak plasma concentrations. Other investigators (*14*) reported that peak plasma concentrations of lidocaine after intravenous administration were significantly increased in patients with congestive heart failure. This was attributed to decreased liver perfusion and a slower rate of lidocaine metabolism. The CV patients also received different drugs preoperatively (that is, isosorbide dinitrate and digitalis) and during induction of anesthesia. These agents might alter the distribution, protein binding, or metabolism of cocaine. We have evidence from in vitro experiments that cocaine is rapidly hydrolyzed in the plasma and that this reaction is inhibited by compounds that inhibit pseudocholinesterase (*15*). Any drugs (for example, succinylcholine) or chronic clinical conditions that alter the activity of this enzyme might theoretically affect the rate of cocaine metabolism.

Because of its brief and intense psychological effects, cocaine is widely believed to be an evanescent drug (*2*). This belief is based to some extent on assumed rapid metabolism and on the reports of street users. Adriani and Campbell (*16*) using the method of Woods *et al.* (*17*) reported that after topical application of cocaine in animals the concentrations in plasma resembled those seen after rapid intravenous injections, with peaks occurring within 4 to 6 minutes and being one-third to one-half those obtained after rapid intravenous injection. However, Woods *et al.* (*17*) measured cocaine plasma concentrations in dogs. They applied cocaine in dosages of 10 to 15 mg per kilogram of body weight by various routes and found peak plasma concentrations of 1000 to 4600 ng/ml. They detected significant concentrations of cocaine in plasma for 10 hours after subcutaneous administration and suggested that this prolonged presence of cocaine in plasma might represent a relative equilibrium between the absorption of cocaine from the subcutaneous depot and detoxification mechanisms. Our dosage was much less and our peak values were all less than the minimum they were able to detect.

Our finding that cocaine persisted in the plasma for 4 to 6 hours was unexpected in view of the reports by street users and by Adriani and Campbell (*16*) but was consistent with the reports of Woods *et al.* (*17*). To ascertain whether this might be caused by prolonged absorption, we obtained swabs (cotton wetted with normal saline) of the nasal mucosa from five patients 1, 2, and 3 hours after cocaine application. These swabs were then eluted and analyzed by thin-layer chromatography (TLC) (*18*). Compounds with the same R_F as cocaine were identified in the nasal swabs taken from these patients at 1, 2, and 3 hours. In two patients the spots were eluted from the TLC plates, and when submitted to GLC yielded a single peak with the same relative retention time as cocaine. The presence of cocaine on the nasal mucosa for 3 hours after application suggested that prolonged absorption of cocaine, perhaps as a result of its vasoconstrictive action, might explain its persistence in plasma. This was consistent with the results of Nayak *et al.* (*19*), who found in rats that, after a 20 mg per kilogram of body weight dose given subcutaneously, there was 27.7 percent of the dose remaining at the site 1 hour after injection and 8.8 percent remaining after 4 hours.

Table 2. Time course of cocaine hydrochloride concentrations in the plasma of dental patients. The values are expressed as means ± standard errors. The numbers in parentheses indicate the number of patients.

Time (min)	Cocaine in plasma (ng/ml)
0	1.8 ± 0.7 (4)
6	46.0 ± 17.3 (3)
10	97.2 ± 10.1 (3)
15	108.6 ± 18.4 (4)
20	126.7 ± 22.7 (4)
60	147.6 ± 18.9 (4)
180	103.5 ± 15.3 (3)
360	59.4 ± 23.0 (3)

The cocaine concentrations in plasma in dental patients (Table 2) peaked at approximately 60 minutes and then decreased in a log-linear fashion over the next 5 hours with an apparent half-life in the plasma of 2.5 hours. Since cocaine remained on the nasal mucosa for 3 hours this probably did not represent a true biologic half-life but rather represented a combination of absorption and elimination. Similarly, Nayak *et al.* (*19*) found that the plasma half-life of cocaine in rats was 4.99 hours after a 20 mg per kilogram of body weight dose was given subcutaneously.

The peak concentrations in plasma occurred later than the time of maximum euphoria reported by street users. These reports indicated peak psychological "highs" within 3 to 5 minutes of application and almost no effect 15 minutes after application. In fact, individuals who abuse cocaine may repeat the dosages every 15 minutes to maintain the psychological effect. In our study, the peak concentrations in plasma occurred about 60 minutes after application, and it is possible that the intense euphoria from cocaine may be re-

lated to the rapidly increasing concentration of cocaine in plasma and not to peak values. Alternatively, since cocaine is highly lipid soluble, the euphoria may better correlate with concentrations at brain receptor sites than with peak concentrations in plasma. Nayak *et al.* (*19*) found significant amounts of cocaine and its active metabolite, norcocaine, in the brains of rats after intravenous (8 mg per kilogram of body weight) and subcutaneous (20 mg per kilogram of body weight) injections.

The development of a toxic psychosis in those individuals who repeat the dosage every 15 minutes might be explained by accumulation secondary to the persistence of cocaine in the plasma for 4 to 6 hours. Since cocaine plasma concentrations may remain elevated during surgical procedures, it might also potentiate sympathomimetic amines given later in the course of such procedures. This possibility should be considered in the clinical use of cocaine.

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6. Seven of the CV patients had coronary artery bypass grafts while one patient had a mitral valve replacement and one patient had both a coronary artery bypass graft and an aortic valve replacement.
7. Three of the dental patients had extractions of their third molars while one dental patient had the excision of a small benign intraoral lesion.
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10. The internal standard was synthesized by overnight refluxing of benzoylecgonine in acidified *n*-propanol, and purified by preparative thin-layer chromatography.
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12. Arterial blood samples were obtained from CV patients and venous blood samples were obtained from dental patients. To ascertain whether this affected our results, we compared simultaneous arterial and venous blood samples in CV patients on two occasions (a 3-minute sample and a 60-minute sample) and found a venous-arterial plasma level ratio of 0.84 and 0.98, respectively. Other investigators [E. Erikssen, S. Englessen, S. Wahlquist, B. Ortengren, *Acta Chir. Scand. Suppl.* **358**, 25 (1966)] found that the peripheral venous-arterial blood concentration ratio for lidocaine was 0.73 ± 0.03 (mean ± S.E.) and for prilocaine was 0.47 ± 0.03. They attributed this difference to the rapid uptake of these local anesthetics by skeletal muscle.
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18. The swabs were eluted with 0.1*N* H_2SO_4. The aqueous phase was adjusted to *p*H 9.6 to 9.8 with carbonate buffer, and extracted with ether. The ether was evaporated and the residue was analyzed by the use of TLC with development in ethyl acetate, methanol, and ammonia (85 : 10 : 5) and sprayed with potassium iodoplatinate.
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Nicotinic Acid Reduction of Plasma Volume Loss After Thermal Trauma

Abstract. *Intravenous administration of nicotinic acid to the anesthetized dog prior to thermal trauma reduced plasma loss at 10 minutes after burn from 7 milliliters per kilogram to less than 2 millimeters per kilogram. During the next 50 minutes plasma loss was the same in treated and untreated animals. An additional dose of nicotinic acid 30 minutes after burn prevented this further loss.*

Increased vascular permeability and plasma volume loss occur rapidly after thermal injury to either humans or animals. Although the loss of plasma was recognized early (*1*), systematic studies of this plasma extravasation are relatively recent (*2*). The physiological mechanisms responsible for this alteration of vascular permeability are not known at present. One or more mediator substances may be released directly as the result of the trauma or indirectly by the activation of the sympathetic nervous system (*3*). In order to test this postulate, we examined a number of pharmacological agents to determine whether it was possible to reduce the loss of plasma volume after thermal injury to experimental animals. The agent which has been most interesting to us has been nicotinic acid.

Mongrel dogs of either sex, weighing between 8 and 15 kilograms, anesthetized with sodium pentobarbital (30 to 35 mg per kilogram of body weight) were used in these studies. Isotopically labeled (^{125}I) albumin was injected into the femoral vein. Arterial samples were collected at timed intervals after injection. The plasma volume at the time of injection was determined by retrograde extrapolation. Plasma volumes were determined prior to burn and at 10 minutes and at 60 minutes after trauma. Three groups of experimental animals were studied. The first and second groups of animals were studied on alternate days and the third group of animals was studied immediately upon completion of study of the first two groups. All animals received a third-degree flame burn over 40 percent of their body area. One

Table 1. Plasma volume loss in mongrel dogs after flame burn. Eight animals received no treatment, six animals received nicotinic acid (10 mg/kg) before burn, and eight animals received nicotinic acid (10 mg/kg) before burn and an additional dose (5 mg/kg) after burn.

Treatment	Plasma volume loss (ml/kg)		
	0 to 10 min after burn	10 to 60 min after burn	0 to 60 min after burn
None	7.11 ± 1.47	4.99 ± 1.87	12.10 ± 1.73
Nicotinic acid*	0.89 ± 1.01†	5.94 ± 1.43	6.83 ± 1.95†
Nicotinic acid‡	1.88 ± 0.41†	0.52 ± 0.71†§	2.40 ± 0.52†§

*10 mg/kg before burn. †Indicates significant difference by unpaired *t*-test from untreated group ($P < .05$). ‡10 mg/kg before burn and 5 mg/kg 30 minutes after burn. §Indicates significant difference by unpaired *t*-test from group receiving 10 mg/kg before burn.