detached from the dish because of handling in the tritium box. At 30 minutes, the [^{3}H]NAD$^+$ pool is comparable in normal and transformed cells, is not saturated, and is nearly one-half the value at 60 minutes (Table 3). Therefore GPDH is not only present (Table 1) and active (Fig. 1) in transformed cells, but the ratio of the activity of the enzymes of the two reactions that produce NAD$^+$, that is, LDH and GPDH, are comparable in normal and transformed cells. Since LDH level (Table 1) and activity (*8*) are higher in transformed cells than in normal cells, the GPDH activity must also be higher to give comparable ratios of the two enzyme activities.

Many postulates have been put forth regarding possible reasons for increased aerobic glycolysis of tumor cells, some of which are no longer accepted (*16*). Boxer and Devlin based theirs on measurement of enzyme activities alone, and their postulate is no longer tenable as a general explanation for increased aerobic glycolysis. There are, of course, changes in many enzyme levels after transformation, such as the rise in LDH. Whether these changes result from rapid growth and nutritional conditions of the culture or whether they are direct consequences of transformation needs further scrutiny.

We have proposed (*9*) that the initial increase in glycolysis that occurs after virus transformation is the result of altered glucose uptake in these cells. An increase in the rate of glucose transport after virus transformation has been demonstrated for chick cells in culture (*17*). The changes in glycolytic enzyme patterns and levels as well as in glycogen synthesis and pentose shunt are then seen as secondary events that may also be produced in normal cells if the level of glucose uptake is varied (*9*).

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References and Notes

1. S. Weinhouse, *Science* **124**, 267 (1956).
2. G. E. Boxer and T. M. Devlin, *ibid.* **134**, 1495 (1961).
3. T. E. Barman, *Enzyme Handbook* (Springer-Verlag, New York, 1969), vol. 1, p. 32.
4. C. E. Wenner, *Adv. Enzymol.* **29**, 321 (1967).
5. A. L. Lehninger, *Biochemistry* (Worth, New York, 1970), p. 409.
6. O. Dionisi, A. Cittadini, T. Gelmazzi, T. Galeotti, T. Terranova, *Biochim. Biophys. Acta* **216**, 71 (1970).
7. T. L. Steck, S. Kaufman, J. P. Bader, *Cancer Res.* **28**, 1611 (1968).
8. M. J. Bissell, C. Hatie, H. Rubin, *J. Natl. Cancer Inst.* **49**, 555 (1972).
9. M. J. Bissell, R. C. White, C. Hatie, J. A. Bassham, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2971 (1973).
10. A. Rein and H. Rubin, *Exp. Cell Res.* **49**, 666 (1968).
11. D. Dolberg and M. J. Bissell, *In Vitro* **10**, 26 (1974).
12. M. J. Bissell, H. Rubin, J. Hatie, *J. Exp. Cell Res.* **68**, 404 (1971).
13. J. A. Bassham, M. J. Bissell R. C. White, *Anal. Biochem.* **61**, 479 (1974).
14. W. A. Rambeck, M. J. Bissell, J. A. Bassham, *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 203 (1975).
15. N. O. Kaplan, in *The Enzymes*, P. D. Boyer, H. A. Lardy, K. Myrbäck, Eds. (Academic Press, New York, 1960), vol. 3, p. 115.
16. J. Paul, in *Cells and Tissues in Culture*, E. N. Willmer, Ed. (Academic Press, London, 1965), pp. 239–276.
17. M. J. Weber, *J. Biol. Chem.* **248**, 2978 (1973); S. Venuta and H. Rubin, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 653 (1973); F. R. Kletzien and J. F. Perdue, *J. Biol. Chem.* **249**, 3375 (1974).
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Demonstration of Kinetoplast DNA in Dyskinetoplastic Strains of *Trypanosoma equiperdum*

Abstract. *4,6-Diamidino-2-phenylindole (DAPI) forms a highly fluorescent complex with DNA which allows detection of mitochondrial DNA (K-DNA) in normal and dyskinetoplastic strains of* Trypanosoma equiperdum. *The K-DNA DAPI complexes in the dyskinetoplastic cells, cells lacking detectable K-DNA by other cytochemical methods, are not restricted to a single region of the organism as in the normal strain, but are seen as a row of particles. These observations support the hypothesis that the K-DNA is retained in dyskinetoplastic cells.*

Mitochondrial DNA of trypanosomatid flagellates is localized in an enlarged region of the mitochondrion, the kinetoplast, where electron microscopy shows that it is organized in a fibrous band. The kinetoplast is readily demonstrable by phase-contrast microscopy in living cells and by Giemsa or Feulgen staining and acridine-orange fluorescence microscopy in fixed cells. In dyskinetoplastic cells, these techniques do not show the kinetoplast; electron microscopy shows that the mitochondrion is still enlarged in the kinetoplast region but the fibrous band of kinetoplast DNA (K-DNA) is replaced by clumps of fibrous material; similar clumps occur at intervals along the length of the mitochondrion. Renger and Wolstenholme (*1*) reported that in both normal and spontaneously dyskinetoplastic strains of *Trypanosoma equiperdum* analytical ultracentrifugation shows the presence of a satellite DNA, buoyant density 1.692, which they identified as K-DNA. I demonstrate here by fluorescence microscopy the presence of K-DNA in living cells of normal and of dyskinetoplastic strains of *T. equiperdum*, using 4,6-diamidino-2-phenylindole (DAPI) as a fluorescent probe.

DAPI was first synthesized as a trypanocidal drug by Dann *et al.* (*2*) and has been shown to bind differentially to yeast mitochondrial and nuclear DNA forming

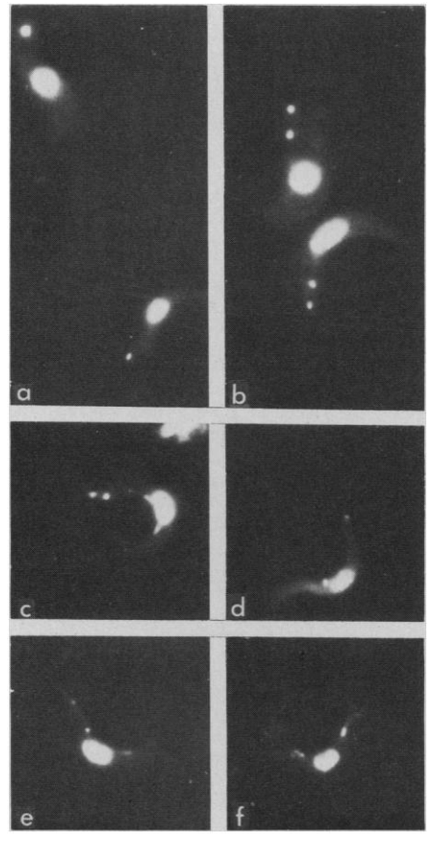

Fig. 1. (a) Photomicrograph of the kinetoplastic strain of *T. equiperdum*, treated with DAPI (0.1 μg/ml) at 37°C for 20 minutes. A single fluorescent nucleus and kinetoplast can be observed in both cells. (b) Photomicrograph of the kinetoplastic strain of *T. equiperdum*, treated with DAPI as in (a). The kinetoplasts in both cells have completed division and the nuclei have not. (c and d) Photomicrographs of the spontaneously dyskinetoplastic strain of *T. equiperdum*, treated with DAPI as above. Fluorescent bodies of varying size can be observed in the cells; the nuclei appear as in the normal cells. (e and f) Photomicrographs of the acriflavin induced dyskinetoplastic strain of *T. equiperdum*, treated with DAPI as above. Distribution of fluorescent bodies in the cells is similar to that seen in the spontaneously dyskinetoplastic cells (×3860).

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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References and Notes

1. H. C. Renger and D. R. Wolstenholme, *J. Cell Biol.* **50**, 533 (1971).
2. O. Dann, G. Bergen, E. Demant, G. Volz, *Ann. Chem.* **749**, 68 (1971).
3. DAPI is specific for duplex DNA; no fluorescence results from addition of DAPI to a solution of single-stranded DNA produced by thermal denaturation and quick cooling. Fluorescence develops and increases in intensity as reannealing proceeds in the solution.
4. W. C. Russell, C. Newman, D. H. Williamson, *Nature (London)* **253**, 461 (1975).
5. S. L. Hajduk, in *Progress in Protozoology: Fourth International Congress of Protozoology, Clermont-Ferrand, France* (1973); P. R. Strauss, *J. Cell Biol.* **53**, 312 (1972).
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