the TATA box and downstream sequences including RNA initiation sites, and the third contains the enhancer element. Our evidence suggests that the LTR is capable of utilizing alternative promoter and/or initiator signals when normal promoter signals are deleted or mutated. While an enhancer element seems to be an absolute necessity for viral gene function, the organization of the retrovirus genome allows the intact 3′ LTR to complement loss of the enhancer element in the 5′ LTR. This versatility of the virus genome with respect to its transcriptional control elements may provide the virus with a strong selective advantage and help to account for its ability to maintain an intimate association with the vertebrate genome.

A. SRINIVASAN
E. PREMKUMAR REDDY
CLAIRE Y. DUNN
STUART A. AARONSON
Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205

References and Notes

1. H. M. Temin, *Cell* **27**, 1 (1981).
2. ———, *ibid.* **28**, 3 (1982).
3. H. E. Varmus, *Science* **216**, 812 (1982).
4. J. L. Manley, A. Fire, A. Cano, P. A. Sharp, M. L. Gefter, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3855 (1982); T. Yamamoto, B. deCrombrugghe, I. Pastan, *Cell* **22**, 787 (1980); M. C. Ostrowski, D. Berard, G. L. Hager, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4485 (1981); S. A. Fuhrman, C. VanBeveren, I. M. Verma, *ibid.*, p. 5411 (1981); S. A. Mitsialis, J. L. Manley, R. V. Guntaka, *Mol. Cell. Biol.* **3**, 811 (1983).
5. G. Khoury and P. Gruss, *Cell* **33**, 313 (1983); Y. Gluzman and T. Shenk, *Enhancers and Eukaryotic Gene Expression* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1983).
6. R. Breathnach and P. Chambon, *Annu. Rev. Biochem.* **50**, 349 (1981); P. Moreau *et al.*, *Nucleic Acids Res.* **9**, 6047 (1981); B. Wasylyk, C. Wasylyk, P. Augereau, P. Chambon, *Cell* **32**, 503 (1983).
7. J. M. Bishop and H. Varmus in *RNA Tumor Viruses*, R. Weiss, N. Teich, H. Varmus, J. Coffin, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), p. 999.
8. A. Srinivasan, E. P. Reddy, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2077 (1981).
9. E. P. Reddy, M. J. Smith, A. Srinivasan, *ibid.* **80**, 3623 (1983).
10. A. Srinivasan, C. Y. Dunn, Y. Yuasa, S. G. Devare, E. P. Reddy, S. A. Aaronson, *ibid.* **79**, 5508 (1982).
11. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
12. C. Benoist and P. Chambon, *Nature (London)* **290**, 304 (1981); P. Gruss, R. Dhar, G. Khoury, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 943 (1981).
13. B. Levinson, G. Khoury, G. F. Vande Woude, P. Gruss, *Nature (London)* **295**, 568 (1982).
14. G. C. Grosveld, E. deBoer, C. K. Shewmaker, R. A. Flavell, *ibid.*, p. 120; P. K. Ghosh, P. Lebowitz, R. J. Frisque, Y. Gluzman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 100 (1981); M. Fromm and P. Berg, *J. Mol. Appl. Genet.* **1**, 457 (1982).
15. T. Shenk, *Curr. Top. Microbiol. Immunol.* **93**, 25 (1981); S. L. McKnight and R. Kingsbury, *Science* **217**, 316 (1982).
16. T. G. Wood, M. L. McGeady, D. G. Blair, G. F. Vande Woude, *J. Virol.* **46**, 726 (1983); D. J. Jolly, A. C. Esty, S. Subramani, T. Friedmann, I. M. Verma, *Nucleic Acids Res.* **11**, 1855 (1983).
17. F. Bolivar, R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, *Gene* **2**, 95 (1977).
18. We thank A. Nguyen, G. Sanders, and H. Fuhrer for help at various stages of the work.

17 October 1983; accepted 1 December 1983

Fluorescence-Line-Narrowed Spectra of Polycyclic Aromatic Carcinogen–DNA Adducts

Abstract. *The laser excited fluorescence-line-narrowed spectrum of DNA modified with (±)-r-7,t-8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo*[a]*pyrene (BPDE), the ultimate carcinogenic metabolite of benzo*[a]*pyrene (BP), has been obtained in a water-glycerol-ethanol glass at 4.2 K. The spectrum was well resolved and highly characteristic of the chromophore. Comparisons were made between the spectrum of this modified DNA and the isolated deoxyguanosine-BPDE adduct and a series of other 7,8,9,10-tetrahydro-BP (THBP) derivatives. 9-Hydroxy-BP 4,5-oxide, which is also involved in the binding of BP to DNA, and THBP have very similar conventional broadband fluorescence spectra. However, the fluorescence-line-narrowed spectra of their derivatives were readily distinguishable either as individual components or as mixtures.*

Many carcinogens, including polycyclic aromatic hydrocarbons (PAH's), require metabolic activation to derivatives that bind covalently to DNA before they exert their carcinogenic effects. Conventional fluorescence spectra of DNA containing such derivatives, obtained at temperatures substantially above 4.2 K and with broadband excitation sources, have shed light on this activation process (*1*). Such spectra have fluorescence line widths of a few hundred reciprocal centimeters, and therefore species with similar structures cannot be distinguished in mixtures. This limitation can be circumvented with the use of fluorescence-line-narrowed (FLN) spectrometry (*2*, *3*), which affords line widths of a few reciprocal centimeters. The direct determination of substitutional PAH isomers in samples, such as solvent-refined coal, has been possible with the use of FLN spectrometry (*4*). This technique would be very valuable in the study of DNA adduct formation resulting from human exposure to PAH mixtures such as diesel engine emissions, cigarette smoke, or coke-oven emissions if FLN spectra could be obtained on DNA samples isolated from cells from such exposed individuals.

As a first step toward this goal, we

Table 1. The FLN spectra of THBP derivatives; λ_{exc}, excitation wavelength. Vacuum-corrected vibrational frequencies (in reciprocal centimeters) were measured relative to the fluorescence origin. Values are accurate to ± 10 cm^{-1}. The following chemicals, THBP (R7–10 = H in Fig. 1), (±)-7-hydroxy-THBP, 10-oxo-THBP, and 9-methoxy-BP 4,5-dihydrodiol, were supplied by R. G. Harvey, University of Chicago. The (±)-10-hydroxy-THBP was obtained by sodium borohydride reduction of the corresponding ketone. The BPDE was obtained through the Chemical Repository of the National Cancer Institute and was used to prepare DNA modified with BPDE (*11*). A side product of the modification reaction was the (±)-BP tetrol (7,8,9,10-tetrahydroxy-THBP). The BPDE-dG (R7–9 = hydroxy, R10 = deoxyguanosine attached through its 2-amino group in Fig. 1) was prepared by enzymic hydrolysis of the modified DNA (*13*). We purified all the derivatives, except the modified DNA, by reverse-phase HPLC, using mixtures of water and methanol. Letters in parentheses, *s*, m, and w, indicate strong (> 0.5), medium (0.5 to 0.2), and weak (< 0.2) peaks, respectively, relative to the most intense. Strengths are assigned qualitatively relative to the most intense vibrational frequency in the spectrum. The frequencies of many of the weak peaks have not been tabulated.

THBP (λ_{exc} = 3784 Å, 156 μ*M*)	7-Hydroxy-THBP (λ_{exc} = 3800 Å, 132 μ*M*)	10-Hydroxy-THBP (λ_{exc} = 3784 Å, 660 μ*M*)	Tetrol (λ_{exc} = 3771 Å, 220 μ*M*)	BPDE-dG (λ_{exc} = 3771 Å, 0.17 μ*M*)	BPDE-DNA (λ_{exc} = 3784 Å, 8 μ*M*)*
284(w), 311(w)	294(w)	270(w)	251(w)	251(w)	242(w)
331(w), 372(w)		467(w)	463(w)	456(w)	445(w)
460(w), 494(w)			551(w)	544(w)	552(w)
574(m)	582(w)	574(w)	592(w)	579(w)	592(w)
636(m)	635(w)	628(w)			
753(m)	759(w)	747(w)	785(m)	785(m)	770(m)
			864(m)	844(m)	836(w)
1072(w)	1069(w)	1072(w)	1073(w)	1069(w)	
1104(m)	1107(m)	1104(m)	1105(m)	1105(m)	1101(m)
1162(w)	1177(w)	1168(w)			
1245(s)	*1240(s)*	*1238(s)*	*1234(s)*	*1234(s)*	*1241(s)*
1270(s)	*1265(s)*	*1264(s)*	1278(m)	1278(m)	1272(m)
1333(w)	1321(w)	1320(w)	1310(w)	1317(w)	
1404(s)	*1408(s)*	*1420(s)*	*1411(s)*	*1404(s)*	*1404(s)*
1551(w)	1550(m)	1545(w)	1547(m)	1547(m)	1546(m)
1595(w)	1581(w)	1558(w)	1587(w)	1580(w)	1583(w)
1625(w)	1624(w)	1619(w)	1630(w)	1624(w)	1602(w)

*With respect to the adduct concentration, 0.4 percent of the bases were modified.

have investigated whether it is possible to obtain FLN spectra on the complex structures related to those produced by metabolic activation of PAH's which then covalently bind to DNA. The underlying physics of FLN spectroscopy is treated in detail elsewhere (*5*) but for completeness we present the following brief discussion. A molecule embedded in an amorphous solid such as glass or polymer typically exhibits at 4.2 K vibronic absorption bandwidths of about 300 cm^{-1}. This is referred to as site-inhomogeneous broadening and is a direct result of the host disorder. Thus, the molecule adopts essentially an infinite number of energetically inequivalent sites resulting in a broad distribution of excitation energies for each and every vibronic transition. Classical broadband excitation results in excitation of all sites and a broad emission spectrum. However, if a laser band (about 1 cm^{-1}) is used for excitation, only a narrow subset (isochromat) of sites is excited. At temperatures near 4.2 K to reduce thermal broadening to less than 1 cm^{-1} and at sufficiently low concentrations to avoid intermolecular electronic energy transfer, dramatic narrowing of the fluorescence spectrum results (FLN).

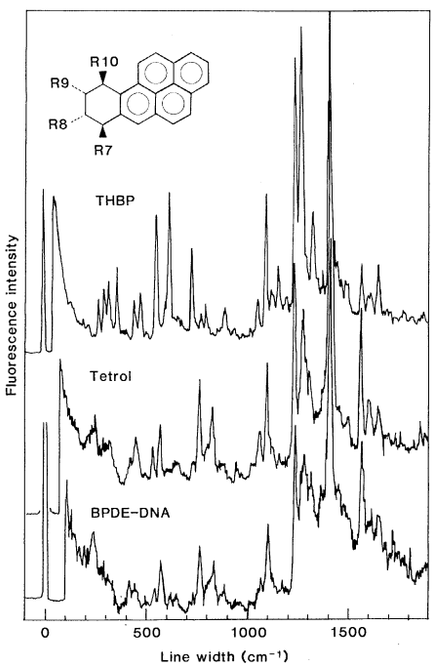


Fig. 1. The FLN spectra were generated with a nitrogen pumped dye laser (*2*). All molecules were embedded in a 10 percent ethanol–60 percent glycerol–40 percent water glass in polystyrene tubes (Falcon, Oxnard, California) and immersed in liquid helium at 4.2 K. The concentrations of THBP, tetrol, and BPDE-DNA (0.4 percent modified) were 156 μM, 220 μM, and 8 μM (adduct), respectively. The resulting fluorescence was dispersed by a 0.3-m scanning monochromator operated with a bandpass of about 6 cm^{-1} so that with a laser line width of 1.6 cm^{-1} the fluorescence line widths are monochromator-limited. The dispersed fluorescence spectrum was normalized to the laser pulse by a dual-gated amplifier (*12*). Derivatives, except the modified DNA, were dissolved in ethanol prior to incorporation into the glass-solvent mixture. The modified DNA was dissolved in 0.5 ml of water and diluted to a volume of 1.1 ml with the ethanol-glycerol components of the glass-forming solvent. The derivatives, in the glass-forming solvent, were inserted into the top of a double-nested liquid helium optical Dewar, which had been evacuated to a pressure of 10^{-6} torr and filled with 3 liters of liquid helium (*2*). After ~ 15 minutes glass formation was complete, and the samples were then lowered into position, below the liquid helium level, in the quartz optical tail section (*8*).

Earlier analytical applications of FLN spectrometry have focused on PAH's and their nonpolar derivatives (*2, 3*). It was observed that the intensity of low-frequency (about 30 cm^{-1}) "phonon" side bands which build on the sharp zero-phonon vibronic lines appears to be enhanced by substitution. In the extreme, the sharp lines can be absent and only the relatively broad (about 100 cm^{-1}) side bands are observed. This results in a marked loss of selectivity and was considered a distinct possibility for the DNA-PAH adducts and PAH metabolites at the outset of this research. We chose benzo[*a*]pyrene (BP), a known carcinogen and potential human health hazard, as a model PAH. Considerable information is available on its metabolic activation (*6*) and the structures of its adducts with DNA (*7*).

In addition to DNA modified with (±)-*r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE), six other compounds, 7,8,9,10-tetrahydrobenzo[*a*]pyrene (THBP), 7,8,9,10-tetrahydroxy-THBP (tetrol), 7-hydroxy-THBP, 10-hydroxy-THBP, deoxyguanosine-BPDE adduct (BPDE-dG), and 9-methoxy-BP 4,5-dihydrodiol (4,5-diol), were investigated by FLN spectrometry. Water-glycerol-ethanol glass (*8*) is a suitable matrix for FLN spectrometry for all seven compounds. Generally, the excitation was chosen to coincide with the origin band or with a low-energy vibronic band of lowest singlet absorption system. Representative spectra are shown in Fig. 1 for BPDE-DNA, THBP, and tetrol. At the resolution of the experiments, the FLN spectrum of BPDE-DNA and BPDE-dG (Table 1) are identical. The data show that there are significant differences in the spectra obtained for the THBP derivatives (Table 1) and pyrene (*2*). However, differences of this magnitude are produced by methylation of pyrene (*8*). The spectra in Fig. 1 and Table 1 were obtained with origin-band excitation so that the resonant fluorescence origins located at 0 cm^{-1} are obscured by laser light scatter. The bands beginning at about 250 cm^{-1} correspond to zero-phonon vibronic transitions terminating at ground-state vibrational sublevels whose frequencies can be directly determined from the ordinate energy scale. The absence of significant phonon side-band activity in Fig. 1 is characteristic of all the compounds studied and noteworthy. Except for 10-hydroxy-THBP and 4,5-diol, which has a chrysene chromophore, the photoexcitation spectra of the compounds in the glass are essentially identical with respect to peak positions and relative intensities.

At this time the potential of FLN spectrometry for high-resolution analysis of mixtures of PAH-DNA adducts is best illustrated by our results for a binary

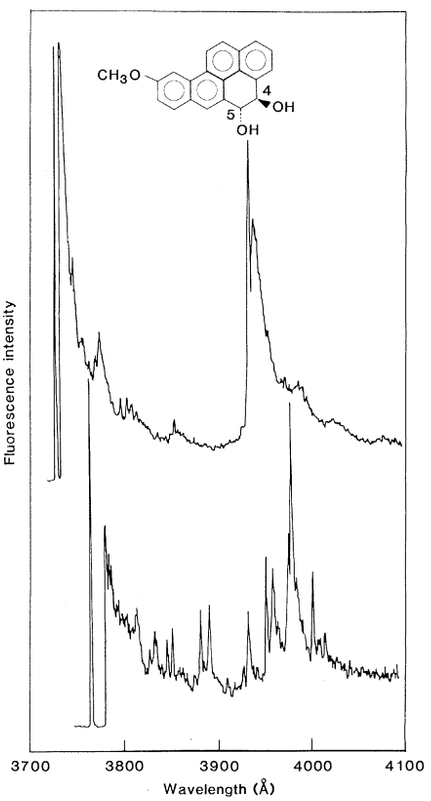


Fig. 2. The FLN spectra of a mixture of 200 μM tetrol and 28-μM 9-methoxybenzo[*a*]pyrene 4,5-dihydrodiol embedded in an ethanol-glycerol-water glass at 4.2 K. For the top spectrum, the excitation wavelength at 3732 Å is selective for 9-methoxybenzo[*a*]pyrene 4,5-dihydrodiol. The frequencies for the emission peaks are 326(w), 487(w), 528(w), 865(w), and 1382(*s*) cm^{-1}, respectively; (s) and (w) have the same significance here as in Table 1. The top spectrum is identical to the standard FLN spectrum of 9-methoxybenzo[*a*]pyrene 4,5-dihydrodiol. In the bottom spectrum, the excitation wavelength at 3762 Å is selective for tetrol (Fig. 1).

mixture of tetrol and 9-methoxy-BP 4,5-diol. It has been reported that the 9-hydroxy-BP 4,5-oxide is a metabolite responsible, at least in part, for the binding of BP to DNA in rat hepatocytes (*9*). It has not been possible to prepare this compound synthetically, and so its 9-methoxy analog has been used. The lowest singlet absorption spectra of the two compounds correspond to that of chrysene and are therefore very similar and blue-shifted relative to the absorption of tetrol. The conventional fluorescence spectra of tetrol, 9-hydroxy-BP 4,5-diol, and 9-methoxy-BP 4,5-diol are essentially identical with emission maxima at 380 and 400 nm. Since the lowest singlet absorption spectra of tetrol and BPDE-DNA are also very similar, the selectivity problem for the tetrol–4,5-diol binary mixture is equivalent to that for a mixture of the adducts of DNA formed with BPDE and 9-hydroxy-BP 4,5-oxide. The FLN spectra of the mixture are shown in Fig. 2. Because the absorption spectra of the two compounds are shifted relative to each other, they can be distinguished by selective excitation. One can see this selectivity by comparing the lower spectrum of Fig. 2 with the tetrol spectrum in Fig. 1. The upper spectrum of Fig. 2 is due entirely to 9-methoxy-BP 4,5-diol. Thus, it is established that, after the isolation of nucleic acids from cells exposed to BP, the BPDE and 9-hydroxy-BP 4,5-oxide adducts could be distinguished by FLN spectrometry. This is a considerable simplification over the best current method for this characterization, which involves extensive enzyme digestion of the DNA, derivitization, and high-performance liquid chromatographic (HPLC) analysis (*10, 11*).

Our results demonstrate that FLN spectra for highly substituted PAH derivatives bound to polymeric materials can be obtained and that fluorescence line narrowing in polar glasses is a promising approach for high-selectivity analysis of DNA obtained after in vivo exposure to PAH mixtures. Such analyses will require improvements in sensitivity, which are possible at this time (*12*).

VOLKER HEISIG
ALAN M. JEFFREY
Cancer Center/Institute for Cancer Research, Division of Environmental Science, and *Department of Pharmacology, Columbia University, New York 10032*
MICHAEL J. MCGLADE
GERALD J. SMALL
Ames Laboratory, Department of Energy, and *Department of Chemistry, Iowa State University, Ames 50011*

References and Notes

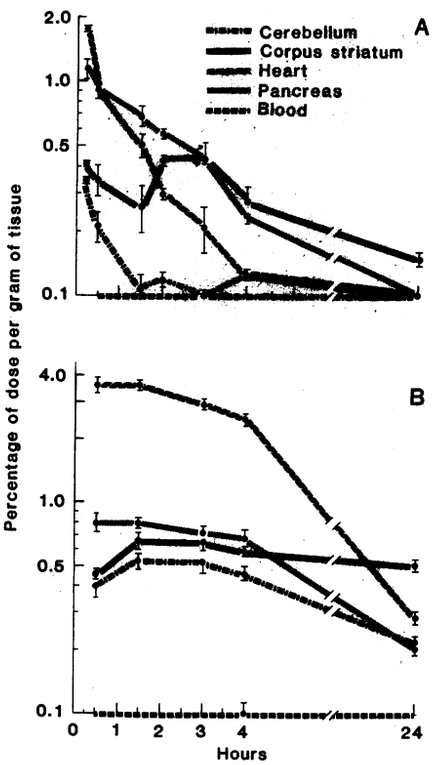
1. P. Daudel, M. Duquesne, P. Vigny, P. L. Grover, P. Sims, *FEBS Lett.* **57**, 250 (1975); V. Ivanovic, N. E. Geacintov, I. B. Weinstein, *Biochem. Biophys. Res. Commun.* **70**, 1172 (1976).
2. J. C. Brown, J. A. Duncanson, Jr., G. J. Small, *Anal. Chem.* **52**, 1711 (1980).
3. I. Chiang, J. M. Hayes, G. J. Small, *ibid.* **54**, 315 (1982); J. M. Hayes, I. Chiang, M. J. McGlade, J. A. Warren, G. J. Small, *Soc. Photo-Opt. Instrum. Eng. J.* **286**, 117 (1981); J. H. Eberly, W. C. McColgin, K. Kawaoka, A. P. Marchetti, *Nature (London)* **251**, 215 (1974).
4. Y. Yang, A. P. D'Silva, V. A. Fassel, *Anal. Chem.* **53**, 894 (1981).
5. R. I. Personov, in *Spectroscopy and Excitation of Condensed Molecular Systems*, V. M. Agranovich and R. M. Hochstrasser, Eds. (North-Holland, New York, 1983), chap. 10.
6. H. V. Gelboin, *Physiol. Rev.* **60**, 1107 (1980).
7. A. M. Jeffrey *et al.*, *Nature (London)* **269**, 348 (1977); *J. Am. Chem. Soc.* **98**, 5714 (1976).
8. J. C. Brown, thesis, Iowa State University (1982).
9. B. Jernstrom, S. Orrenius, O. Undeman, A. Groslund, A. Ehrenberg, *Cancer Res.* **38**, 2600 (1978).
10. V. Heisig, R. Santella, C. Cortez, R. G. Harvey, A. M. Jeffrey, *Proc. Am. Assoc. Cancer Res.* **24**, 68 (1983).
11. K. W. Jennette *et al.*, *Biochemistry* **16**, 932 (1977).
12. M. J. McGlade, thesis, Iowa State University (1983).
13. H. Brown, A. M. Jeffrey, I. B. Weinstein, *Cancer Res.* **39**, 1673 (1979).
14. Supported by National Cancer Institute grant CA 021111 and Department of Energy contract W-7405-eng-82, Office of Health and Environmental Research.

26 August 1983; accepted 22 November 1983

External Imaging of Cerebral Muscarinic Acetylcholine Receptors

Abstract. *A radioiodinated ligand that binds to muscarinic acetylcholine receptors was shown to distribute in the brain by a receptor-mediated process. With single-photon-emission imaging techniques, radioactivity was detected in the cerebrum but not in the cerebellum, whereas with a flow-limited radiotracer, radioactivity was detected in cerebrum and cerebellum. Single-photon-emission computed tomography showed good definition of the caudate putamen and cortex in man.*

The muscarinic acetylcholine receptor (m-AChR) appears to play an essential role in many physiological and behavioral responses (*1*). Sleep, avoidance behavior, learning, and memory are thought to be mediated by m-AChR. A decrease in m-AChR density has been observed in elderly patients, those with Huntington's chorea, and those with Alzheimer's dementia. Ethanol, barbiturates, and antidepressants as well as chronic exposure to insecticides, may also affect the concentration of the m-AChR (*2, 3*). Most of these observations have been made in autopsy studies in humans or in animal studies. None of



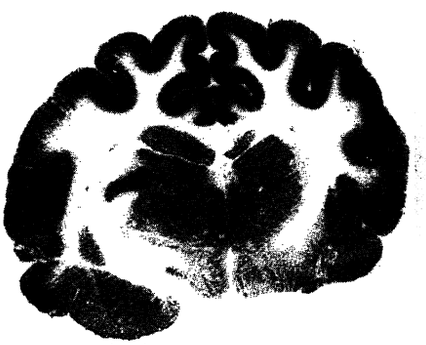


Fig. 1 (left). (A) Distribution of (*R*)-4-[^{125}I]IQNB in Sprague-Dawley rats as a function of time after femoral vein injection. (B) Distribution of (*R*)-[^{3}H]QNB in Sprague-Dawley rats as a function of time after femoral vein injection. The average value and the standard error for six rats are shown at each time point. Fig. 2 (right). Autoradiograph of (*R*)-4-[^{125}I]IQNB in a cat 15 minutes after injection. Results after 2 hours were similar. Coronal section, 20 μm thick; 500 μCi of the radioiodinated material was injected intravenously under light anesthesia (Innovar); the animal was killed by barbiturate injection and frozen (Freon and dry ice); and the brain was embedded in carboxymethyl cellulose. Exposure time, 10 days on SB5 film.