whether these changes occur as a result of the transport of aromatic hydrocarbons into membranes or as a result of the lipids adsorbing onto the particulates. This adsorption process is likely to cause disruption of the spherical bilayer. We investigated the integrity of the vesicles in the presence of particulates by two means. The first involved release of the fluorogenic probe, umbelliferyl phosphate, from the internal volume of the vesicles (*14*). Umbelliferyl phosphate is only weakly fluorescent, whereas the hydrolysis product, umbelliferone, is highly fluorescent. The negative charges on umbelliferyl phosphate prevent diffusion across lipid bilayers, and this material cannot escape unless the bilayers are disrupted. The fluorogenic probe was trapped in the internal volume of dimyristoyl-L-$\alpha$-lecithin vesicles by sonication, and the external probe was removed by dialysis. Alkaline phosphatase was added to the external media, rapidly cleaving any umbelliferyl phosphate that was released. The vesicles were disrupted by boiling, then more alkaline phosphatase was added which produced a large increase in fluorescence intensity. None of the particulates used in these studies caused any disruption of the phospholipid vesicles.

The second method we used to examine vesicle integrity in the presence of particulates depended on both the ability of the lanthanides to shift the frequencies of nuclear magnetic resonances and the impermeability of intact lipid bilayers to these triply charged ions. Europium chloride, when added to preformed lecithin vesicles, binds only to the external phosphates. The proximity of $Eu^{3+}$ to the outer surface choline residues results in a shift in their resonance frequency (*15*). Disruption of the lipid bilayer results in the inner and outer shifts becoming equivalent. None of the particulates used in this study affected the europium-induced separation of the inner and outer choline resonances. We conclude that particulate contact with dimyristoyl and dipalmitoyl lecithin vesicles was not disruptive in our studies.

This study demonstrates the feasibility of using fluorescence spectroscopy for measuring the exchange of PAH between particulates and cell membranes. The sensitivity of the fluorescent properties of these aromatic hydrocarbons to their local environment should allow the preparation of particulates with known surface distributions of carcinogens. The study of coated particulates should prove useful in understanding how the mode of carcinogen administration to experimental animals affects the cellular availability and carcinogenicity. In addition, such knowledge should provide a rational basis for understanding the synergistic effects of polynuclear aromatic hydrocarbons and particulates.

J. R. LAKOWICZ
M. MCNAMARA
L. STEENSON

*Freshwater Biological Institute, University of Minnesota, Navarre 55392*

### References and Notes

1. I. J. Selikoff, E. C. Hammond, J. Churg, *J. Am. Med. Assoc.* **204**, 104 (1968).
2. E. C. Hammond and I. J. Selikoff, *IARC (Int. Agency Res. Cancer) Sci. Publ.* **8**, 312 (1973).
3. T. J. Haley, *J. Pharm. Sci.* **64**, 1435 (1975).
4. V. Saffiotti, F. Cefis, L. H. Kolb, *Cancer Res.* **28**, 104 (1968).
5. L. N. Pylev and L. M. Shabad, *IARC (Int. Agency Res. Cancer) Sci. Publ.* **8**, 99 (1973).
6. L. N. Pylev, *Bull. Exp. Biol. Med. (USSR)* **52**, 1316 (1961).
7. S. Mondal and C. Heidelberger, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 219 (1970).
8. J. S. Harrington, *Ann. N.Y. Acad. Sci.* **132**, 31 (1965).
9. H. L. Falk, P. Koten, I. Markal, *Cancer* **11**, 482 (1958).
10. H. L. Falk, A. Miller, P. Koten, *Science* **127**, 474 (1958).
11. D. M. Jerina and J. W. Daly, *ibid.* **185**, 573 (1974).
12. J. B. Birks and J. W. Cameron, *Proc. R. Soc. London Ser. A* **249**, 297 (1959).
13. Fluorescence spectra were obtained with a spectrofluorometer (SLM Instruments). Samples were placed in a cuvette (2 by 2 cm) which in turn was placed in a cuvette holder equipped with a magnetic stirrer and a thermostat. The samples could thus be observed while the particles were kept in suspension by continuous stirring. Scattered light at the excitation wavelength did not affect our measurements because we used the appropriate optical filtration. The excitation wavelength was 305 nm, and the excitation and emission band-passes were 8 and 2 nm, respectively. Phospholipid vesicles were prepared by sonication of lipid in buffer, followed by centrifugation at 48,000*g* for 1 hour. Unless otherwise stated, all experiments were conducted at 40°C. The buffer was 0.01*M* tris, 0.05*M* KCl, *p*H 7.5. Umbelliferyl phosphate (Polysciences), as the sodium salt, was incorporated into vesicles by cosonication of phospholipid and umbelliferyl phosphate, followed by dialysis to remove the external fluorophore. Dimyristoyl-L-$\alpha$-lecithin and dipalmitoyl-L-$\alpha$-lecithin (both from Sigma) were used without further purification. The *N*-ethylcarbazole, chrysene, and 1,6-diphenylhexatriene were from Aldrich. The *N*-ethylcarbazole was recrystallized six times from absolute ethanol, and the chrysene was recrystallized from benzene. Porous glass was obtained from Corning (200 to 350 $m^2/g$), glass beads (3 to 8 $\mu$m) from Polysciences, acid-washed alumina from Merck, and silica gel G preparative thin-layer plates from Analabs. *N*-Ethylcarbazole was coated onto the particulates from ethanol, 1,6-diphenylhexatriene from acetone, and chrysene from benzene; the solvent was then removed under vacuum with continual mixing. Silica with adsorbed chrysene was prepared by chromatographing chrysene on preparative thin-layer plates, with cyclohexane and benzene (80:20 by volume) as the solvent system; this was followed by mechanical removal of the chrysene band. The fluorophore content of the particulates was determined by solvent extraction and subsequent optical density measurements.
14. H. R. Six, W. W. Ulmura, S. C. Kinsky, *Biochemistry* **13**, 4050 (1974).
15. R. Lawaczeck, M. Kainosho, S. I. Chan, *Biochim. Biophys. Acta* **443**, 313 (1976).
16. We thank the Freshwater Biological Research Foundation and especially its founder, R. Gray, Sr., without whose assistance this work would not be possible. We also acknowledge support of NIH grant ES GM 01283-01A1. This work was supported by grant BC-261 from the American Cancer Society and in part by funds contributed by the American Heart Association. The technical assistance of D. Hogen was invaluable for the progress of this research. J.R.L. is an established investigator of the American Heart Association.

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## $\beta$-Glucuronidase Catalyzed Hydrolysis of Benzo[*a*]pyrene-3-Glucuronide and Binding to DNA

Abstract. *$\beta$-Glucuronidase catalyzes the hydrolysis of benzo*[a]*pyrene-3-glucuronide to 3-hydroxybenzo*[a]*pyrene. During the enzymatic hydrolysis, a benzo*[a]*pyrene derivative is formed which binds to DNA to a far greater extent than either the 3-hydroxybenzo*[a]*pyrene or its glucuronide. These results suggest that conjugates of benzo*[a]*pyrene may be converted by $\beta$-glucuronidase at intracellular and organ sites distal to the initial sites of oxygenation and conjugation of benzo*[a]*pyrene to activated intermediates that are possibly carcinogenic.*

Benzo[*a*]pyrene is a carcinogenic hydrocarbon commonly found in the environment (*1*). The toxic, mutagenic, and carcinogenic effects of this hydrocarbon are dependent on metabolism by the mixed function oxidases and metabolically related enzymes (*2*). Benzo[*a*]-pyrene is enzymatically converted to epoxides and at least four phenols and three quinones. The epoxides are also converted to dihydrodiols (*3*). The oxygenated metabolites are also converted to glutathione (*4*), glucuronide (*5*), and sulfate conjugates (*6*). Recent studies have demonstrated the metabolic formation of a diol epoxide of benzo[*a*]pyrene, *r*-7,*trans*-8-dihydroxy-*trans*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene which is highly mutagenic (*7*) and binds very actively to nucleic acids (*8, 8a*).

The water-soluble conjugates of benzo[*a*]pyrene, such as the glucuronides, have been generally viewed as detoxification products. These products, largely formed in the liver, are transported systemically to remote organs and are excreted in the urine or feces (*9*). When benzo[*a*]pyrene is administered to animals it is excreted in the bile, feces, and urine in the form of water-soluble conjugates, many of which have been identified as glucuronides and sulfates (*9*). Wa-


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ter-soluble conjugates are also formed in cell culture (*9*). We recently demonstrated that glucuronide conjugates are formed from four phenol and two dihydrodiol metabolites of benzo[*a*]pyrene (*5*). One of the latter, the 7,8-dihydrodiol, is the precursor of the highly active diol epoxide (*7*). This report relates to the possible biological activity of the glucuronide conjugates. β-Glucuronidase (E.C. 3.2.1.31) is widely distributed in animal tissues. The enzyme is found in liver, kidney, and spleen, and has a wide distribution in secretory tissues such as mammary gland and thyroid and in intestinal juice, bile, and salivary secretions (*10*). This study was designed to test the possibility that the benzo[*a*]-pyrene glucuronides may not be entirely detoxification products but rather may be converted by glucuronidase to carcinogens at sites distal to their formation.

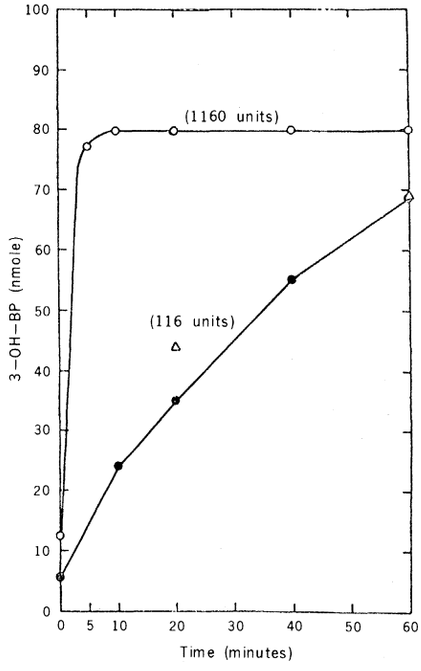


Fig. 1. The hydrolysis of BP-3GA by β-glucuronidase. The reaction mixture contained 100 nmole of BP-3GA, 1160 or 116 Fishman units of β-glucuronidase, and 150 μg of calf thymus DNA in 1.0 ml of 0.1*M* acetate buffer (*p*H 5.0). The mixture was incubated with gentle shaking at 37°C for the times indicated. The reaction was stopped by the addition of 1 ml of acetone and 3 ml of hexane. The mixture was incubated with shaking at 37°C for 10 minutes. A 1.0-ml portion of the organic phase was extracted with 3 ml of 1*N* NaOH. The concentration of extracted 3-OH-BP in the alkali phase was determined spectrophotofluorometrically with activation at 396 nm and fluorescence at 522 nm in an Aminco-Bowman spectrofluorometer. A standard solution of 3-OH-BP was used to make the calibration curve. The triangles show the velocity of hydrolysis by 116 units of β-glucuronidase when the reaction mixture contained 10 percent methanol.

In this study we found that β-glucuronidase converts benzo[*a*]pyrene-3-glucuronide to 3-hydroxybenzo[*a*]pyrene (3-OH-BP) and during this process an intermediate is formed which is covalently bound to DNA. Figure 1 shows the kinetics of hydrolysis of synthetic benzo[*a*]pyrene-3-glucuronide by β-glucuronidase. Two concentrations of enzymes were used. In the presence of 1160 Fishman units of β-glucuronidase the glucuronide rapidly hydrolyzes, the reaction reaching completion at 10 minutes. The recovery of 3-OH-BP from the conjugate was 80 percent. With one-tenth as much enzyme, 116 units, hydrolysis was nearly linear, reaching 70 percent hydrolysis after 60 minutes of incubation. Figure 1 also shows that the presence of small amounts of methanol used in the subsequent binding experiments had no effect on the hydrolysis.

Table 1 shows that in the absence of enzyme there is a low level of binding of 3-OH-BP to DNA. Thus, one benzo-[*a*]pyrene nucleus is bound per 13,000 nucleotides. The presence of β-glucuronidase reduced this binding by half, possibly by nonspecific binding of the enzyme protein to 3-OH-BP. In the absence of β-glucuronidase there is only negligible binding of $^3$H from [$^3$H]-benzo[*a*]pyrene-3-glucuronide to DNA. However, when 116 units of β-glucuronidase were added there was a large stimulation of the binding of a benzo[*a*]pyrene derivative to DNA. Thus 358 μmole of benzo[*a*]pyrene equivalents are bound per mole of DNA phosphorus, or one benzo[*a*]pyrene nucleus per 2800 nucleotides. The binding to DNA was not via free 3-OH-BP and was not related to the rate of release of free 3-OH-BP from the glucuronide. Thus the addition of 80 nmole of 3-OH-BP added at zero time, or five sequential additions of 16 nmole of 3-OH-BP yielded essentially the same amount of binding to DNA. This amount was only one-tenth that observed in the presence of benzo[*a*]pyrene-3-glucuronide and β-glucuronidase. The free 3-OH-BP has been reported to be not carcinogenic (*10, 11*) and only weakly mutagenic (*7, 12*). Of 11 phenols tested for carcinogenicity only the 2-phenol and 11-phenol have reported carcinogenicity (*11, 13*) but these have not been identified as metabolites. Our studies suggest the possibility that the conjugated metabolites may be a precursor of DNA-binding carcinogenic intermediates.

The *O*-glucuronide of *N*-hydroxy-*N*-2-fluorenyl-acetamide is a metabolite of the carcinogen *N*-2-fluorenylacetamide and has been implicated in the binding of the fluorenyl residue to nucleic acids in vivo (*14*). When the *O*-glucuronide of the *N*-hydroxy-*N*-2-fluorenyl-acetamide was deacetylated the *O*-glucuronide of the *N*-2-fluorenyl-hydroxylamine gave fluorenyl-substituted nucleic acid adducts (*15, 16*). These studies suggested that the glucuronide of this carcinogen may be formed in the liver and transported to the urinary bladder. The hydrolysis of the glucuronides to *N*-hydroxy-arylamines followed by their conversion to arylnitrenium ions in the acidic urine was suggested as a mechanism of tumor induction in the bladder by this class of compounds (*16*).

It is possible the C-*O*-glucuronides of polycyclic hydrocarbons are also carcinogenic intermediates. Recent studies have indicated that a specific metabolic product of benzo[*a*]pyrene, a 7,8-diol-9,10-epoxide may be a major carcinogenic form of benzo[*a*]pyrene. This com-

Table 1. Binding of [$^3$H]benzo[*a*]pyrenol-3-glucuronide ([$^3$H]BP-3GA) to DNA and the effect of β-glucuronidase. System a, in total volume of 1 ml (0.1*M* acetate buffer, *p*H 5.0, including 10 percent methanol), contained 100 nmole of tritiated 3-OH-BP (222 mc/mmole) and 150 μg of calf thymus DNA. System b contained, in addition to the contents listed for system a, 116 Fishman units of β-glucuronidase (Sigma). Systems c and d contained 80 nmole of [$^3$H]BP-3GA (40 mc/mmole) instead of [$^3$H]3-OH-BP. The cold glucuronide was chemically synthesized (*20*) and was mixed with metabolically generated glucuronide (*5*). Reaction mixtures were incubated at 37°C for 60 minutes by gentle shaking. The reactions were stopped by the addition of 1 ml of 0.1*M* acetate buffer, *p*H 5.0, and 2 ml of buffer-saturated phenol. The DNA was extracted by shaking at room temperature for 30 minutes and was obtained in 2 ml of aqueous solution after centrifuging at 10,000 rev/min for 1 hour. Absolute ethanol (6 ml) was added to the DNA solution, which was stored in the refrigerator overnight. The amorphous sediment (DNA) was collected by centrifuging at 1500 rev/min for 30 minutes. The DNA was redissolved in 2 ml of acetate buffer and resedimented in ethanol; it was extracted repeatedly with ethanol and purified by repeating the above procedure until there was no radioactivity in the extract. The extent of binding of the BP derivatives to DNA was determined as described previously, with Aquasol being used instead of Liquifluor.

| System | [$^3$H]-3-OH-BP | [$^3$H]-BP-3GA | β-Glucuronidase | Ratio of BP to DNA (μmole/mole P) |
|---|---|---|---|---|
| a | 100 | | | 75 |
| b | 100 | | + | 36 |
| c | | 80 | | 14 |
| d | | 80 | + | 358 |

pound has been identified as a major benzo[*a*]pyrene metabolite bound in vivo to DNA (*7, 8, 8a*). With microsomes, however, other metabolites such as the K-region epoxide and the 9-phenol are also bound to DNA (*8a, 17*). Furthermore, a detailed study of the binding of benzo[*a*]-pyrene in vivo to the DNA of different tissues has not been reported. Our findings that β-glucuronidase catalyzes the binding of benzo[*a*]pyrene-3-glucuronide suggest the possibility that the latter type of metabolite may be an important intermediate of benzo[*a*]pyrene carcinogenesis. It could function as a carrier of a benzo[*a*]pyrene metabolite to distal sites such as the bladder, kidney, or intestines where glucuronidase action may induce its binding to DNA. Renwick and Drasar (*18*) have reported the bacterial hydrolysis of benzo[*a*]pyrene conjugates by gut bacteria and have suggested that this may be a mechanism of retoxification of the polycyclic hydrocarbons. Our studies suggest that the hydrolysis of phenols or diols may yield a reactive intermediate that binds to DNA and be carcinogenic. Another possibility is that the 7,8-diol glucuronide is transported either intracellularly or to a distal organ site where it can be hydrolyzed to the diol and serve as a substrate for diol epoxide formation. Recent reports have indicated a nuclear mixed function oxidase activity which may be responsible for this activation (*19*).

NADAO KINOSHITA*
HARRY V. GELBOIN†
*Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014*

### References and Notes

1. Committee on Biological Effects of Atmospheric Pollutants, *Particulate Polycyclic Organic Matter* (National Academy of Sciences, Washington, D.C., 1972).
2. H. V. Gelboin, N. Kinoshita, F. J. Wiebel, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1298 (1972).
3. A. H. Conney, E. C. Miller, J. A. Miller, *J. Biol. Chem.* **228**, 753 (1957); P. Sims, *Biochem. Pharmacol.* **16**, 613 (1968); N. Kinoshita, B. Shears, H. V. Gelboin, *Cancer Res.* **33**, 1937 (1973); J. K. Selkirk, R. G. Croy, P. P. Roller, H. V. Gelboin, *ibid.* **34**, 3474 (1974); I. V. Wang, J. F. Rasmussen, T. T. Crocker, *Biochem. Biophys. Res. Commun.* **49**, 1142 (1972); E. Boyland and K. Williams, *Biochem. J.* **94**, 190 (1965).
4. N. Nemoto and H. V. Gelboin, *Arch. Biochem. Biophys.* **170**, 739 (1975); ———, W. H. Habig, J. N. Ketley, W. B. Jakoby, *Nature (London)* **255**, 512 (1975).
5. N. Nemoto and H. V. Gelboin, *Biochem. Pharmacol.* **25**, 1221 (1976); N. Nemoto and S. Takayama. *ibid.*, in press; N. Kinoshita and H. V. Gelboin, in preparation.
6. G. M. Cohen, S. M. Haws, B. P. Moore, J. W. Bridges, *Biochem. Pharmacol.* **25**, 2561 (1976).
7. A. Borgen, T. T. Crocker, R. E. Rasmussen, I. Y. Wang, *J. Med. Chem.* **16**, 502 (1973); S. K. Yang, D. W. McCourt, P. R. Roller, H. V. Gelboin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2594 (1976); C. Malaveille, H. Bartsch, P. L. Grover, P. Sims, *Biochem. Biophys. Res. Commun.* **66**, 693 (1975); E. Huberman, L. Sachs, S. K. Yang, H. V. Gelboin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 607 (1976); D. R. Thakker, H. Yagi, A. Y. H. Lu, W. Levin, A. H. Conney, D. M. Jerina, *ibid.*, p. 3381.
8. P. Sims, P. L. Grover, A. Swailand, K. Pal, A. Hewer, *Nature (London)* **252**, 326 (1974); H. W. S. King, M. H. Thompson, P. Brookes, *Cancer Res.* **35**, 1263 (1975); P. Daudel, M. Daquesne, P. Vigny, P. L. Grover, P. Sims, *FEBS Lett.* **57**, 250 (1975); I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, C. Harris, H. Autrup, H. Kasai, K. Nakanishi, *Science* **193**, 592 (1976); A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, K. Nakanishi, *J. Am. Chem. Soc.* **98**, 5714 (1976).
8a. H. W. S. King, M. H. Thompson, P. Brookes, *Int. J. Cancer* **18**, 339 (1976).
9. F. Weigert and J. C. Mottram, *Cancer Res.* **6**, 102 (1946); I. Berenblum and R. Schoental, *ibid.*, p. 699; K. H. Harper, *Br. J. Cancer* **12**, 121 (1958); *ibid.*, p. 645; H. L. Falk, P. Kotin, S. S. Lee, A. Nathan, *J. Natl. Cancer Inst.* **28**, 699 (1962); L. N. Andrianov, G. A. Belitsky, O. J. Ivanova, A. Y. Khesina, S. S. Khitrova, L. M. Shabad, J. M. Vasiliev, *Br. J. Cancer* **21**, 566 (1967); L. Diamond, C. Sardet, G. H. Rothblat, *Int. J. Cancer* **3**, 838 (1968); M. Duncan, P. Brookes, A. Dipple, *ibid.* **4**, 813 (1969); H. Vadi, P. Moldeus, J. Capdevila, S. Orrenius, *Cancer Res.* **35**, 2083 (1975); L. W. Wattenberg and J. L. Leong, *ibid.* **30**, 1922 (1970).
10. M. Wakabayashi, in *Metabolic Conjugation and Metabolic Hydrolysis*, W. H. Fishman, Ed. (Academic Press, New York, 1970), vol. 2, p. 519.
11. J. L. Hartwell and P. Shubik, *Survey of Compounds Which Have Been Tested for Carcinogenic Activity*, PHS Publ. No. 149 (Government Printing Office, Washington, D.C., ed. 2, 1951); P. G. Wislocki, J. Kapitulnik, W. Levin, H. Yagi, P. M. Dansette, D. M. Jerina, A. H. Conney, *Proc. Am. Assoc. Cancer Res.* **18**, 140 (1977).
12. P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, H. Yagi, O. Hernandez, P. W. Dansette, D. M. Jerina, A. H. Conney, *Cancer Res.* **36**, 3350 (1976).
13. J. Kapitulnik, W. Levin, H. Yagi, D. M. Jerina, A. H. Conney, *ibid.*, p. 3625.
14. C. C. Irving, in *Metabolic Conjugation and Metabolic Hydrolysis*, W. H. Fishman, Ed. (Academic Press, New York, 1970), vol. 1, p. 53; C. C. Irving and L. T. Russell, *Biochemistry* **9**, 2471 (1970).
15. R. A. Cardona and C. M. King, *Biochem. Pharmacol.* **25**, 1051 (1976).
16. F. F. Kadlubar, J. A. Miller, E. C. Miller, *Cancer Res.* **37**, 805 (1977).
17. W. M. Baird, R. G. Harvey, P. Brookes, *Cancer Res.* **35**, 207 (1975).
18. A. G. Renwick and B. S. Drasar, *Nature (London)* **263**, 234 (1976).
19. E. G. Rogan, P. Mailander, E. Cavalieri, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 457 (1976); J. M. Pezzuto, M. A. Lea, C. S. Yang, *Cancer Res.* **36**, 3647 (1976); B. Jernstrom, H. Vadi, S. Orrenius, *ibid.*, p. 4107; K. Alexandrov, P. Brookes, H. W. S. King, M. R. Osborne, M. H. Thompson, *Chem. Biol. Interact.* **12**, 269 (1976); H. W. S. King, M. H. Thompson, E. M. Tarmy, P. Brookes, R. G. Harvey, *ibid.* **13**, 349 (1976b).
20. M. Mori and N. Kinoshita, in preparation.

* Present address: Chemistry Department, School of Health Sciences, Kyushu University 90, Maidashi, Higashi-ku, Fukuoka 812, Japan.
† Reprint requests should be addressed to H.V.G.

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## Pineal Serotonin *N*-Acetyltransferase Activity: Abrupt Decrease in Adenosine 3′,5′-Monophosphate May Be Signal for "Turnoff"

Abstract. *Dispersed pinealocytes have been used to study the role of adenosine 3′,5′-monophosphate (cyclic AMP) in the "turnoff" of* N*-acetyltransferase activity. Activity was first stimulated 100-fold by treating cells with* l*-norepinephrine.* l*-Propranolol acted stereospecifically to rapidly reverse this, resulting in a 70 percent loss of enzyme activity within 15 minutes. An even more rapid* l*-propranolol-induced decrease in cyclic AMP also occurred. This together with the observation that the inhibitory effect of* l*-propranolol on* N*-acetyltransferase was blocked by dibutyryl cyclic AMP and phosphodiesterase inhibitors indicate that an abrupt decrease in cyclic AMP may be the signal for the rapid decrease in pineal* N*-acetyltransferase activity.*

One of the rapid transmitter-regulated changes in enzyme activity is the decrease [halving time ($t_{1/2}$), 3 minutes] in rat pineal serotonin *N*-acetyltransferase activity which occurs when an animal is exposed to light after a period of darkness at night (*1*). A similar "turnoff" of enzyme activity occurs at night as a result of *l*-propranolol treatment and when adrenergically stimulated pineal glands are treated with this blocking agent in vitro (*2, 3*). Although it is clear that the adrenergically induced increase in *N*-acetyltransferase activity is mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (*3-6*), this second messenger has not been implicated in either the maintenance of increased *N*-acetyltransferase activity or in the rapid "turnoff" of enzyme activity.

We have examined this problem using a new pinealocyte culture method (*7*) and a sensitive technique for the assay of cyclic AMP (*8*), and here report that the rapid *l*-propranolol-induced decrease in *N*-acetyltransferase activity is accompanied by an even more rapid decrease in cellular cyclic AMP. This and associated observations provide evidence that the signal for "turnoff" of pineal *N*-acetyltransferase activity may be the rapid decrease in cyclic AMP to a critical low level.

Cells isolated from pineal glands of 2-day-old rats were incubated under control conditions for 24 hours. During this period a small degree of aggregation was observed; essentially no cells were attached to the plastic culture tubes. Addition of *l*-norepinephrine (1 μ*M*) resulted in a 100-fold increase in cyclic AMP and *N*-acetyltransferase activity (Fig. 1). The increase in cyclic AMP peaked at about 15 minutes and gradually decreased during the next few hours. The rate of increase of *N*-acetyltransferase activity


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