Although a linear relation can be expected for the spread of subthreshold potentials between A and B when the C group is quiescent, the relation when the C group is active will be complex. The inhibition and disinhibition will both be somewhat delayed. Saturation will occur when the C group is completely blocked, as may have happened in the experiment of Fig. 1b, and nonlinearities could arise both in excitation of the C group and in its effects on cell B; a further factor in the input-output relation will be the effect of the C group back on cell A. Finally, synaptic inputs may modulate electrotonic spread between cells A and B.

Synaptic relations similar to those illustrated in Fig. 3 are found elsewhere in the *Navanax* buccal ganglia; subthreshold depolarizations of the expansion-controlling motoneurons excite neurons that inhibit them (*6*), but the effects are much weaker than in the circumferential neurons. Also, in the stomatogastric ganglion of the spiny lobster there are neurons connected both electrotonically and by inhibitory synapses (*7*). In one instance inhibition apparently causes the cells to fire out of phase when both are excited; however, during prolonged excitation the cells come to fire more or less synchronously, perhaps due to fatigue of the inhibition. Aside from this transition, no phenomena similar to the change in sign of effective coupling were noted. In addition, it was suggested that uncoupling produced by inhibition might be important in this preparation. The circuitry of Fig. 3 could account for the gating phenomenon described by Murray (*8*) in the cerebral ganglia of *Navanax*. In this case subthreshold polarizations in certain cells affect electrotonically mediated transmission of impulses from one set of cells to another set that have not been recorded from intracellularly. The cells that Murray calls gate cells could be electrotonically coupled to cells like the C group that inhibit the postsynaptic elements.

The flexibility of the circuitry described here may allow mediation and perhaps initiation of different patterns of movement involving the same neurons. When the C group neurons are inactive and not excited by the circumferential neurons, the latter cells are coupled typically and tend to fire synchronously. When activity of the C group is modulated by the circumferential neurons, the relation between circumferential cells is transformed into a different mode and the sign of effective coupling between them is reversed. Very large hyperpolarizations of circumferential neurons result from activity in expansion neurons, and post-hyperpolarization firing may operate in initiating circumferential contractions for swallowing. The hyperpolarizations in the circumferential neurons would also alter C group excitability. The pharynx sucks in food and swallows it by peristalsis or rejects it, depending on the circumstances (*9*). Because circumferential neurons control overlapping areas of the pharyngeal wall, synchronous and sequential activation will cause different kinds of movements. Understanding how the different patterns of movement are controlled seems feasible in this simple system.

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References and Notes

1. M. V. L. Bennett, in *Structure and Function of Synapses*, G. D. Pappas and D. P. Purpura, Eds. (Raven, New York, 1972), p. 221; *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **50**, 58 (1972).
2. M. E. Spira and M. V. L. Bennett, *Brain Res.* **37**, 294 (1972).
3. T. J. Carew and E. R. Kandel, *Science* **192**, 150 (1976).
4. High-Mg solutions contained the following ionic concentrations (millimoles per liter): Na^+, 223; K^+, 9; Ca^{2+}, 9.3; Mg^{2+}, 250; Cl^-, 700, and SO_4^{2-}, 25.5. They were buffered with bicarbonate to *p*H 7.8.
5. S. B. Kater, *Am. Zool.* **14**, 3 (1974); C. Sotelo, R. Llinás, R. Baker, *J. Neurophysiol.* **37**, 541 (1974).
6. H. Levitan, L. Tauc, J. P. Segundo, *J. Gen. Physiol.* **55**, 484 (1970).
7. B. Mulloney and A. I. Selverston, *J. Comp. Physiol.* **91**, 1, 53 (1974); D. M. Maynard and A. I. Selverston, *ibid.* **100**, 161 (1975).
8. M. J. Murray, *ibid.* **103**, 189 (1975).
9. R. T. Paine, *Veliger* **6**, 1 (1963); M. S. Woollacott, *J. Comp. Physiol.* **94**, 69 (1974); D. C. Spray, M. E. Spira, M. V. L. Bennett, in preparation.
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Interindividual Variation in Binding of Benzo[*a*]pyrene to DNA in Cultured Human Bronchi

Abstract. *The binding of benzo[*a*]pyrene to DNA in cultured human bronchus was measured in specimens from 37 patients. The binding values ranged from 2 to 151 picomoles of benzo[*a*]pyrene per milligram of DNA with an overall mean ± standard error of 34.2 ± 5.2. This 75-fold interindividual variation in the binding of benzo[*a*]-pyrene to DNA is similar in magnitude to that found in pharmacogenetic studies of drug metabolism. Aryl hydrocarbon hydroxylase is also inducible by benz[*a*]anthracene in the bronchial mucosa.*

Experimental systems are being developed to study the process of carcinogenesis in human tissues which are targets of environmental chemical carcinogens (*1*). The human bronchus, for example, is exposed to such carcinogens as polynuclear aromatic hydrocarbons (PAH) (*2*), which are present in the environment because of the incomplete combustion of fossil fuels and because of tobacco smoke (*3*). The carcinogenic PAH are procarcinogens requiring enzymatic activation into metabolites which bind covalently to cellular macromolecules (*4*). In some studies (*5*) a positive correlation has been found among binding of the PAH to DNA and their carcinogenicity. Whether this correlation will be found in studies of carcinogenesis in man is not known.

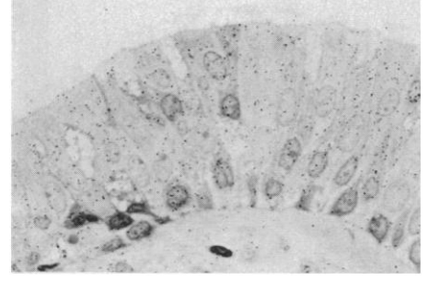


Fig. 1. Section of cultured human bronchus showing three major cell types (mucous, ciliated cells, and basal cells). The cells are well-preserved, and are overlaid with autoradiographic grains after incubation of the bronchi with tritiated BP (*7*).

Human bronchi maintained in explant culture have the capability to activate carcinogenic PAH into metabolites which bind to DNA (*6*). This binding is dependent on temperature of incubation, duration of exposure to PAH, and concentration of PAH (*7*). By means of an autoradiographic assay, it has been shown that in the bronchial mucosa the epithelial cells bind more PAH than the fibroblasts. 7,8-Benzoflavone (*7, 8*), an inhibitor of aryl hydrocarbon hydroxylase (benzopyrene hydroxylase; E.C. 1.14.14.2), decreased the binding of a PAH, benzo[*a*]pyrene (BP), to DNA.

Since the human population is genetically very heterogeneous and the metabolic activation of PAH may be under genetic control, we have studied the interindividual variation in the binding of BP to DNA in cultured human bronchial mucosa.

Human bronchial specimens were ob-

tained either at surgery or immediately after death from patients with and without lung cancer (*9*). The mean age of the cancer patients was 53 years (range 20 to 79) compared to 36 years of the patients without cancer (range 14 to 59). Four to six explants (1 by 1 cm) of grossly normal-appearing bronchi from each patient were cultured in a chemically defined medium (CMRL-1066 containing, per milliliter, 0.1 μg of hydrocortisone hemisuccinate, 1 μg of crystalline bovine insulin, 0.1 μg of β-retinyl acetate, 100 units of penicillin G, and 100 μg of streptomycin) on a rocker platform in an atmosphere of 50 percent O_2, 45 percent N_2 and 5 percent CO_2 as previously described (*10*). After the explants had been cultured for 7 days, tritiated BP (40 μc/ml; 1.5 μM BP; 25 c/mmole; Amersham/Searle) dissolved in dimethylsulfoxide (final concentration 0.5 percent) was added to the culture medium for 24 hours. For morphological studies, bronchial specimens were fixed overnight in 2.7 percent glutaraldehyde buffered by 0.1*M* *s*-collidine, *p*H 7.4, and then processed for examination by high-resolution light microscopy (*7*). As before (*3*), the bronchial epithelium was consistently well preserved (Fig. 1). For biochemical studies, the bronchial mucosa was scraped from the supporting connective tissue of the other bronchial specimens, highly purified DNA was isolated from the cells on CsCl gradients, and the radioactivity bound to DNA was determined as described (*6, 7*). Previous studies in which the conditions were similar and ^{14}C-labeled BP was substituted for ^{3}H-labeled BP demonstrated both that the bound radioactivity was not due to tritium exchange and that the intraindividual variation due to experimental methodology was small (coefficient of variation, 0.1) (*7*).

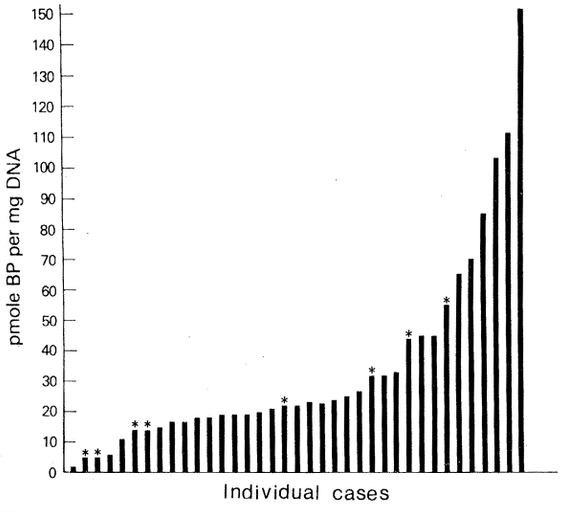
Table 1. Induction of the enzyme aryl hydrocarbon hydroxylase by benz[*a*]anthracene in cultured human bronchus. Bronchial explants were cultured in a chemically defined medium for 7 days; then benz[a]anthracene (10 μg/ml; dissolved in dimethylsulfoxide) was added for 24 hours. The culture medium was replaced with that containing [^{3}H]benzo[a]pyrene (1.5 μ*M*; 20 c/m*M*) for either 4 or 8 hours. Aryl hydrocarbon hydroxylase activity, which was determined by measuring the formation of tritiated water by the method of Hayakawa and Udenfriend (*17*), is expressed as disintegrations per minute per microgram of DNA.

Time of incubation (hours)*	Treatment prior to incubation	Enzyme activity (dpm/μg DNA)
4	Benz[*a*]anthracene	42 ± 0.2
4	Control	13 ± 1.0
8	Benz[*a*]anthracene	128 ± 4.0
8	Control	95 ± 3.0

*Incubation was with [^{3}H]benzo[*a*]pyrene.

The binding levels of BP to DNA in cultured bronchial mucosal cells from the 37 individual cases studied to date is shown in Fig. 2. The values range from 2 to 151 pmole of BP per milligram of DNA with a ratio of 75.5 and a coefficient of variation of 0.94. The overall mean value ± standard error was 34.2 ± 5.2; (for 29 patients with lung cancer the mean value was 36.9 ± 6.6, and for eight patients without lung cancer, 24 ± 6.3). The frequency distribution of the binding values observed is highly skewed (that is, there is a long tail) with the mode being about 17 to 19 and the median being 22.5. While this frequency distribution appears to be unimodal (Fig. 3), more patients will have to be studied to determine whether there are human subpopulations that have different binding levels of BP. It is unlikely that this interindividual variation was caused by differences in levels of aryl hydrocarbon hydroxylase induced by exogenous factors, for example, tobacco smoke, in the patients studied; to allow induced levels of aryl hydrocarbon hydroxylase to reach basal levels, the bronchial specimens were cultured for 7 days prior to the addition of ^{3}H-labeled BP. As monitored by high-resolution light microscopy, viability of the bronchial epithelium was good; however, subtle changes in cellular physiology could, in part, account for some of the observed differences in the binding of BP to macromolecules. In addition to maintaining good cellular morphology and being capable of metabolizing BP to intermediates that bind to DNA, the cultured bronchial mucosa has aryl hydrocarbon hydroxylase activity which is induced by benz[*a*]anthracene (Table 1).

While the binding of chemical carcinogens to DNA in human tissues has not been previously compared among individuals, the genetic control of aryl hydrocarbon hydroxylase has been studied in both human and mouse cells. Nebert and Gielen, (*11*) have shown that the inducibility of this microsomal enzyme in the liver varies among different strains of mice. They concluded that the induction of aryl hydrocarbon hydroxylase by PAH in mice is expressed as a simple autosomal dominant trait. However, the degree of inducibility appears to be tissue-specific. Wiebel *et al.* (*12*) found that in strains of mice in which hepatic aryl hydrocarbon hydroxylase is not induc-



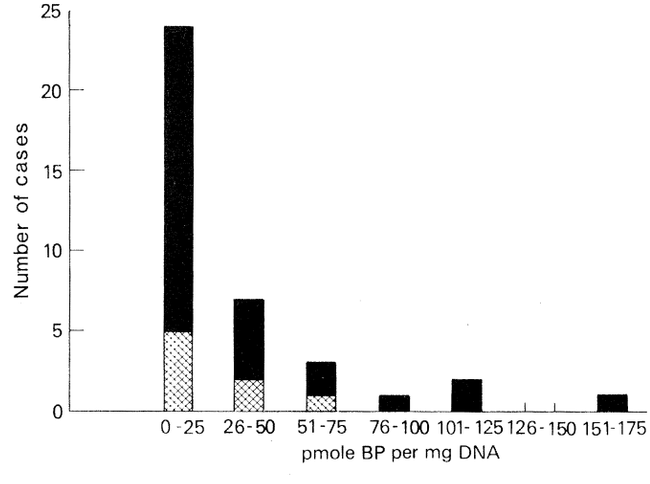


Fig. 2 (left). The interindividual variation in the binding levels of BP to DNA in cultured human bronchi. The values marked with an asterisk are from patients without lung cancer and the remainder are from lung cancer patients. Fig. 3 (right). The distribution frequency of binding levels of BP to DNA in cultured bronchi. The cross-hatched areas represent patients without lung cancer.

ible, aryl hydrocarbon hydroxylase may be induced in the lung, kidney, and skin. In addition, Burki *et al.* (*13*) studied induction of aryl hydrocarbon hydroxylase in explants of fetal mouse liver. Their data suggest that its induction is under multifactorial genetic control. Therefore, the exact inheritance of aryl hydrocarbon hydroxylase induction in mice remains controversial.

The genetic control of aryl hydrocarbon hydroxylase in human cells and tissues is also uncertain. Induction of aryl hydrocarbon hydroxylase by 3-methylcholanthrane has been studied in cultured human lymphocytes (*14*). In this study of 353 healthy individuals, both constitutive and 3-methylcholanthrane–induced levels of aryl hydrocarbon hydroxylase were measured. Inducibility varied from 1.3- to 4.5-fold among individuals, with three subpopulations consisting of individuals with low, intermediate, and high inducibility being evident. Analysis of the data suggested a single locus of genetic control with gene frequences of low and high alleles being 0.717 and 0.283, respectively. In a preliminary study (*15*), patients with lung cancer appeared to have a higher frequency of both intermediate and high inducibility than that of the patients serving as controls. These findings await confirmation.

The level of aryl hydrocarbon hydroxylase has also been measured in human placenta: a 70-fold variation of aryl hydrocarbon hydroxylase among women who smoked 15 to 20 cigarettes daily was observed (*16*). This variation is similar in magnitude to the variation in the level of BP bound to DNA in the study reported here. The possibility that factors such as drug intake, tobacco smoking, or occupation may have influenced our results is now being investigated.

The possibility exists that subpopulations of humans may be unusually susceptible to procarcinogens because of an enhanced genetically determined capability to activate these chemical carcinogens metabolically. This hypothesis requires further study (*1*).

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References and Notes

1. For a review, see C. Harris, *Beitr. Pathol.* **158**, 389 (1976).
2. J. Fraumeni, *Proceedings of the XI International Cancer Congress* (Excerpta Medica, Amsterdam, 1974), vol. 3, p. 327.
3. Committee on Biological Effects of Atmospheric Pollutants, *Particulate Polycyclic Organic Matter* (National Academy of Sciences, Washington, D.C., 1972); U.S. Department of Health, Education, and Welfare, *The Health Consequences of Smoking: A Report to the Surgeon General* (Government Printing Office, Washington, D.C., 1971), p. 265.
4. H. Gelboin, F. Wiebel, N. Kinoshita, in *Chemical Carcinogenesis*, P. Ts'o and J. DiPaolo, Eds. (Dekker, New York, 1974), p. 311.
5. C. Heidelberger, *Annu. Rev. Biochem.* **44**, 79 (1975).
6. C. Harris, V. Genta, A. Frank, D. Kaufman, L. Barrett, E. McDowell, B. Trump, *Nature (London)* **252**, 68 (1974).
7. C. Harris, A. Frank, C. van Haaften, D. Kaufman, R. Connor, F. Jackson, L. Barrett, E. McDowell, B. Trump, *Cancer Res.* **36**, 1011 (1976).
8. C. Harris, H. Autrup, R. Connor, A. Frank, L. Barrett, E. McDowell, B. Trump, *Proceedings of the International Symposium on the Detection and Prevention of Cancer* (Excerpta Medica, Amsterdam, in press).
9. The distribution of bronchial specimens obtained at surgery was: (i) three patients (two black males and one black female) without lung cancer, and (ii) 29 patients (19 white males, nine black males, and one white female) with lung cancer. The bronchial specimens obtained at immediate autopsy were all from five patients (three white males and two black males) without cancer who died from head trauma. A retrospective analysis of hospital charts was done to determine the history of tobacco smoking for all of the patients; 28 of the lung cancer patients were smokers, one of the noncancer patients was a nonsmoker, and no information was listed for the remaining patients. Among the smokers, no correlation was found between the number of cigarettes smoked (number of packs per day times years smoked) and the amount of BP bound to DNA in cultured bronchial mucosa. Because of the incomplete listing of information concerning smoking, occupation, diet, and medication in hospital charts, this information is now being obtained for use in further studies.
10. B. Trump, E. McDowell, L. Barrett, A. Frank, C. Harris, in *Experimental Lung Cancer, Bioassay and Carcinogenesis*, E. Karbe and J. Park, Eds. (Springer-Verlag, New York, 1974); E. McDowell, A. Frank, C. Harris, B. Trump, *Cancer Res.* **36**, 1003 (1976).
11. D. Nebert and J. Gielen, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1315 (1972).
12. F. Wiebel, J. Leutz, H. Gelboin, *Arch. Biochem. Biophys.* **154**, 292 (1973).
13. K. Burki, A. Liebelt, E. Bresnick, *ibid.* **158**, 641 (1973).
14. G. Kellermann, M. Luyten-Kellermann, C. Shaw, *Am. J. Human Genet.* **25**, 327 (1973).
15. G. Kellermann, C. Shaw, M. Luyten-Kellermann, *N. Engl. J. Med.* **289**, 934 (1973).
16. A. H. Conney and W. Levin, *Chemical Carcinogenesis Essays, IARC Scientific Publication No. 10*, R. Montesano and L. Tomatis, Eds. (International Agency for Research on Cancer, Lyon, 1974), p. 3.
17. T. Hayakawa and S. Udenfriend, *Anal. Biochem.* **51**, 501 (1973).

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25-Hydroxyvitamin D_3: Autoradiographic Evidence of Sites of Action in Epiphyseal Cartilage and Bone

Abstract. *Tritiated 25-hydroxyvitamin D_3 was administered to growing rats to morphologically document its sites of action. Highly selective incorporation occurred in epiphyseal hypertrophic cells, epiphyseal matrix, osteoid, osteoblasts, and osteocytes of metaphyseal bone spicules. The labeled metabolite appeared in chondrocytic lacunar matrix coincident with hypertrophic cell death as evidenced by histological examination. The tritiated 25-hydroxyvitamin D_3 became localized only in areas of active mineralization.*

The major metabolites of vitamin D_3, 25-hydroxycholecalciferol [25-(OH)D_3] and 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$], have been shown to be active forms of the parent vitamin in the promotion of bone resorption (*1, 2,*), possibly by acting as permissive agents for the action of parathyroid hormone (*3*). The calcium-transporting function of such metabolites has usually been studied in the intestine (*4*). Recently, attention has turned to the binding of 25-(OH)D_3 and 1,25$(OH)_2D_3$ in mineralized tissues (*5*) where these metabolites participate in promoting cell-mediated bone calcium mobilization (*6*). As early as 1956 it was demonstrated that bones which included epiphyseal growth plates concentrated significant amounts of ^{14}C-labeled vitamin D (*7*). However, no direct histological evidence documents the effects of 25-(OH)D_3 on bone or cartilage, and the possible sites of action of 25-(OH)D_3 in bone, such as in the epiphyseal growth plate undergoing endochondral ossification (a site of active mineralization and mineral mobilization), have not been differentiated. Although it is known that 25-(OH)D_3 exerts a direct effect on epiphyseal cartilage metabolism (*8*), the cellular and extracellular sites of incorporation of this metabolite in calcifying connective tissues have not been demon-

Table 1. The incorporation of 25-(OH)[^{3}H]D_3 in growing rat bone and epiphyseal cartilage, as revealed by autoradiography. Each number represents the average of counts taken from eight separate areas of each respective region.

Epiphyseal zone or cell population	Grains per cell (No.)	Grains per unit area of matrix (No.)
Resting zone	0	0
Proliferating zone	2	1
Hypertrophic zone	18*	4
Provisional calcification	9†	38
Osteoblasts	13	36‡
Osteocytes	15	28
Osteoclasts	0	4

*Viable cells. †Nonviable cells. ‡Osteoid.