tant in more fluctuating environments (*3*). Arctic soils experience considerably less seasonal temperature fluctuation than those of the temperate zone (*7*). Populations and species from arctic tundra have significantly lower ($P < .05$) acclimation potentials, when rates are measured at 20°C, than species from more fluctuating environments (Table 1). A similar latitudinal trend in acclimation potential was observed when rates were measured at 5°C (*7*).

Latitudinal trends in the phosphate absorption capacity and the acclimation potential of phosphate absorption indicate that this physiological process has adapted to local temperature regimes ranging from warm, thermally fluctuating soils to cold, stable soils. Further studies, particularly in the tropics, will be necessary to separate the selective influence of temperature fluctuation from that of temperature per se.

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References and Notes

1. P. F. Scholander, W. Flagg, V. Walters, L. Irving, *Physiol. Zool.* **26**, 67 (1953); C. L. Prosser and F. A. Brown, *Comparative Animal Physiology* (Saunders, Philadelphia, ed. 2, 1961); G. N. Somero and P. W. Hochachka, *Am. Zool.* **11**, 157 (1971).
2. H. A. Mooney and W. D. Billings, *Ecol. Monogr.* **31**, 1 (1961); S. J. McNaughton, *Science* **150**, 1829 (1965); O. Björkman, *Britonia* **18**, 214 (1966); L. G. Klikoff, *Nature (Lond.)* **212**, 529 (1966).
3. W. D. Billings, P. J. Godfrey, B. F. Chabot, D. P. Bourque, *Arct. Alp. Res.* **3**, 277 (1971).
4. S. I. Scholander and J. T. Kanwisher, *Plant Physiol.* **34**, 574 (1959); H. A. Mooney, R. D. Wright, B. R. Strain, *Am. Midl. Nat.* **72**, 281 (1964).
5. B. F. Chabot and W. D. Billings, *Ecol. Monogr.* **42**, 163 (1972).
6. H. A. Mooney and F. Shropshire, *Oecol. Plant.* **2**, 1 (1967); P. L. Tobiessen, *Diss. Abstr.* **32**, 1507B (1970).
7. F. S. Chapin, thesis, Stanford University (1973).
8. M. Fried and H. Broeshart, *The Soil-Plant System in Relation to Inorganic Nutrition* (Academic Press, New York, 1967); C. A. Black, *Hortic. Sci.* **4**, 314 (1969); R. L. Bieleski, *Annu. Rev. Plant Physiol.* **24**, 225 (1973).
9. Procedure B [E. Epstein, W. E. Schmid, D. W. Rains, *Plant Cell Physiol.* **4**, 79 (1963)].
10. A. Läuchli, *Int. J. Appl. Radiat. Isot.* **20**, 265 (1969).
11. E. Epstein, *Mineral Nutrition of Plants: Principles and Perspectives* (Wiley, New York, 1972); P. Nissen, *Psychol. Plant.* **28**, 113 (1973).
12. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
13. F. S. Chapin, *Proceedings 1972 Tundra Biome Symposium*, S. Bowen, Ed. (U.S. Tundra Biome, Fairbanks, Alaska, 1972), p. 46.
14. The acclimation potential is analogous to Q_{10} (increase in the rate of a chemical reaction for each 10°C increase in temperature) but compares rates measured at the same temperature and acclimated to different temperatures.
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Plasma Dopamine β-Hydroxylase: A Possible Aid in the Study and Evaluation of Hypertension

Abstract. *The activity of dopamine β-hydroxylase (DBH) in plasma ranged from 2 to 100 units per liter of plasma in 82 apparently healthy subjects (ages 22 to 35 years). A nonnormal pattern of distribution was evident: 62 subjects had values below 35 units (18 ± 1), while 13 of the remaining 20 subjects had values above 60 units (80 ± 5). Those with low DBH activity had lower values for urinary catecholamine excretion (31 ± 3 micrograms), with normal and stable blood pressure; those with high DBH activity had higher values for urinary catecholamine excretion (72 ± 6 micrograms), with greater lability of arterial blood pressure. The DBH activity was significantly elevated in patients with labile (74 ± 2 mm-Hg) or fixed (57 ± 2 mm-Hg) essential hypertension. The results indicate that plasma DBH activity is low and that it falls within a narrow range in young adults with normal and stable blood pressure.*

Dopamine β-hydroxylase (DBH) (E.C. 1.14.2.1), the enzyme that converts dopamine to norepinephrine, is present in the synaptic vesicles of postganglionic sympathetic neurons (*1*). The release of norepinephrine from the nerve terminal appears to occur via the process of exocytosis, an event that is also accompanied by the simultaneous release of the soluble portion of DBH (*2*). For this reason, it has been proposed that plasma DBH activity may serve as an index of the activity of the sympathetic nervous system (*3*). Thus far, however, the successful application of measurements of DBH activity has been restricted by the wide range of values that has thus far been described in supposedly normal subjects, and by the large degree of overlap between these values and those observed in certain disease states (*4, 5*). Parallel measurements of the plasma and urinary concentrations of catecholamines have exhibited a similar degree of overlap and scatter (*6*).

Our study was undertaken to define the range of plasma DBH activity more exactly in apparently healthy subjects, and to establish its relation to the quantitative excretion of total urinary catecholamines and the day-to-day lability of blood pressure. Blood was collected in heparinized tubes via venipuncture of the antecubital vein; the tubes were cooled in ice, and the blood sample was then centrifuged for 10 minutes. Activity of DBH in plasma was measured in 82 apparently healthy subjects (ages 22 to 35 years) (*4*). The results are shown in Table 1. The values ranged from 2 to 100 international units and did not have a normal pattern of distribution as judged by a chi-square test for goodness of fit ($P < .001$). Sixty-two subjects (76 percent) had values below 35 unit/liter [mean: 18 ± 1 (S.E.M.) unit/liter], while 13 of the remaining 20 subjects (16 percent) had values above 60 unit/liter (mean: 80 ± 5 unit/liter). It should be noted that 53 of the 62 values in the first group actually fell below 25 units per liter of plasma. The observed pattern of distribution suggested that more than one population might be included within this group of apparently healthy subjects.

Table 1. Distribution of plasma dopamine β-hydroxylase activities, expressed as international units (micromoles per minute) per liter of plasma at 37°C in a group of control subjects.

Activities (I.U.)		*N*	% Total *N*
Range	Mean ± S.E.M.		
2–100 (total)	31 ± 3	82	100
2–35*	18 ± 1	62	76
36–59	48 ± 2	7	9
60–100†	80 ± 5	13	16

* In this group 53 of the 62 values were below 25 (low DBH group). † High DBH group.

Accordingly, further studies were carried out in five subjects from the low DBH group (plasma DBH activity less than 25 unit/liter) and six subjects from the high group (plasma DBH activity greater than 60 unit/liter). Blood pressure was evaluated by the auscultatory method in the supine and upright positions between 9 and 10 a.m. for seven consecutive days. Blood samples were obtained on days 2 and 5, and 24-hour collections of urine were obtained on day 2.

Urinary concentrations of norepinephrine and epinephrine were determined fluorometrically (*7*) and expressed as micrograms per gram of creatinine (*8*). Comparison with creatinine minimizes the influence of individual variation in lean body mass and the completeness of urine collections (*9*), but it does not

Table 2. Relation between plasma dopamine β-hydroxylase activity, urinary catecholamines, and blood pressure. The statistical significance of differences between the two groups of subjects was established by the two-tailed *t*-test. The DBH activities are expressed as international units (micromoles per minute) per liter of plasma at 37°C. Catecholamines (CA) are expressed as micrograms of CA per gram of creatinine per 24 hours. Blood pressures and changes were measured in millimeters of mercury.

Subjects	DBH (I.U.)		Catecholamine	Δ Systolic BP range/ Δ Diastolic BP range	BP readings >130/85 (No.)
	Day 2	Day 5			
			From 2 to 25 range in Table 1		
M.T.	10	9	19	20/20	0
W.S.	18	23	34	10/15	0
B.J.	12	12	40	50/10*	1*
R.H.	18	14	28	28/20	0
G.M.	12	11	33	20/10	0
Mean ± S.E.M.					
	14 ± 1	14 ± 2	31 ± 3	26 ± 5/15 ± 2	0.2 ± 0.2
			From 60 to 100 range in Table 1		
K.H.	99	86	84	55/30	4
R.N.	57	70	55	40/35	4
B.B.	89	79	98	40/45	5
L.B.	79	82	74	60/45	8
L.K.	66	72	67	35/20	5
L.F.	61	63	52	45/20	6
Mean ± S.E.M.					
	75 ± 6	75 ± 3	72 ± 6	46 ± 3/33 ± 4	5.3 ± 0.6
	$P < .001$	$P < .001$	$P < .001$	$P < .01/P < .005$	$P < .001$

* First reading was high systolic.

allow for possible variation in the renal clearance of catecholamines (*10*) (Table 2). DBH activity was constant from day to day in each individual, but those whose DBH activity was high exhibited values for urinary catecholamines that were at least twice those of individuals whose DBH activity was low. The correlation between plasma DBH activity and urinary catecholamines was excellent; the linear coefficient of correlation, *r*, was .93, as determined by linear regression analysis. These data suggest that each of these parameters reflect sympathetic nervous system activity. Finally, in subjects with high DBH activity, it was noted that the systemic arterial blood pressure (systolic/diastolic) varied widely from day to day, the average variation being (46 ± 3)/(33 ± 4) mm-Hg. Each of these individuals exhibited, on the average, a blood pressure greater than 130/85 mm-Hg on five occasions. In contrast, the systemic blood pressure exhibited much less lability from day to day in subjects with low DBH activity [average: (26 ± 5)/(15 ± 2) mm-Hg], and values above 130/85 mm-Hg were not observed (Table 2).

Labile or fluctuating arterial blood pressures occur in apparently healthy subjects as well as in patients with hypertension (*10, 11*). The observed correlation between the presence of high plasma DBH activity and greater lability of arterial blood pressure (often exceeding a value of 130/85 mm-Hg) suggested that measurements of plasma DBH activity might be useful in the evaluation of patients with hypertension. As a test of this hypothesis, plasma DBH activity was determined in 29 patients with hypertension who had been referred to the Duke University Medical Center for evaluation. The DBH activity was highest [average: 65 ± 3 (S.E.M.) units] in those patients who were thought to exhibit fixed or labile essential hypertension, whereas it was much lower (average: 15 ± 3 units) in those patients with secondary forms of hypertension (due to primary aldosteronism, renal parenchymal disease, renal arterial stenosis, adrenal disease) (Table 3). DBH activity was even low in patients with increased plasma renin activity due to renal vascular disease (*12*). These observations suggest that measurements of plasma DBH activity may prove useful to the clinical separation of primary from secondary forms of hypertension.

Table 3. Plasma dopamine β-hydroxylase activity in patients with hypertension. Numbers in parentheses indicate the number of patients; I.U., international units.

Hypertensive category	Plasma DBH activity* (I.U.)
Labile (6)	74 ± 2*
Essential (6)	57 ± 2*
Renal (parenchymal and vascular) (11)	15 ± 3
Miscellaneous (6)	16 ± 5

* $P < .001$, different from the three other groups.

Studies utilizing a double-isotope derivative assay for plasma catecholamines have suggested that the plasma concentration of catecholamines may be elevated in patients with labile or fixed essential hypertension (*13*). Such an observation suggests that increased activity of the sympathetic nervous system may play a role in the genesis of certain forms of hypertension. Our data are consistent with this hypothesis. Furthermore, the finding of higher values for DBH activity in apparently healthy subjects with labile blood pressure of insufficient degree to warrant the clinical diagnosis of labile hypertension, and the observation of similar values for DBH activity in patients with definitive labile and fixed hypertension are consistent with the thesis of Eich *et al.* (*14*) that labile hypertension may represent an early phase in the development of fixed essential hypertension. Eich *et al.* described a group of young people with fluctuating arterial blood pressure, increased cardiac output, and normal peripheral resistance who showed, a few years later, a syndrome of sustained diastolic hypertension with normal cardiac output and increased peripheral resistance (*14*). Although more complete study is necessary, it appears that measurements of plasma DBH activity will prove useful in the diagnostic evaluation of patients with various types of hypertension as well as provide the means to further our understanding of the physiological mechanisms involved in these diseases.

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References and Notes

1. L. T. Potter and J. Axelrod, *J. Pharmacol. Exp. Ther.* **142**, 299 (1963); W. P. De Potter, A. D. Smith, A. F. De Schaepdryver, *Tissue Cell* **2**, 529 (1970).
2. O. H. Viveros, L. Arqueros, N. Kirshner, *Life Sci.* **7**, 609 (1968); L. B. Geffen, B. G. Livett, R. A. Rush, *J. Physiol.* **204**, 593 (1969); G. P. Gewirtz and I. Kopin, *Nature (Lond.)* **227**, 406 (1970); A. D. Smith, W. P. De Potter, E. J. Moerman, A. F. De Schaepdryver, *Tissue Cell* **2**, 547 (1970).
3. R. Weinshilboum and J. Axelrod, *Circ. Res.* **28**, 307 (1971); M. Goldstein, L. S. Friedman, A. C. Bohuon, F. Guernot, *N. Engl. J. Med.* **286**, 1123 (1972); G. F. Wooten, R. Eldridge, J. Axelrod, R. S. Stern, *ibid.* **288**, 284 (1973).
4. T. Nagatsu and S. Udenfriend, *Clin. Chem.* **18**, 980 (1972).
5. L. S. Freedman, T. Chuchi, M. Goldstein, F. Axelrod, I. Fish, J. Dancis, *Nature (Lond.)* **236**, 310 (1972).
6. P. J. Nestel and A. E. Doyle, *Australas.*

Ann. Med. **17**, 295 (1968); P. J. Nestel and M. D. Esler, *Circ. Res.* **27**, II-75 (1970); K. Engelman, B. Portnoy, A. Sjoerdsma, *ibid.*, p. I-141.
7. U. S. von Euler and F. Lishajko, *Acta Physiol. Scand.* **51**, 348 (1961).
8. K. D. G. Edward and H. M. Whyte, *Aust. J. Exp. Biol.* **36**, 383 (1958).
9. ———, *Clin. Sci.* **18**, 361 (1959).
10. J. H. Windesheim, G. M. Roth, E. A. Hines, Jr., *Circulation* **11**, 878 (1955).
11. M. D. Esler and K. J. Goulston, *N. Engl. J. Med.* **288**, 16 (1973); A. E. Doyle and F. H. Smirk, *Circulation* **12**, 543 (1955).
12. R. Stone, S. M. Schanberg, J. C. Gunnells, R. R. Robinson, N. Kirshner, in preparation.
13. G. Pickering, *Am. J. Med.* **52**, 570 (1972); W. J. Louis, A. E. Doyle, S. Anavekar, *N. Engl. J. Med.* **228**, 599 (1973); V. de Quattro and S. Chan, *Lancet* **1972-I**, 806 (1972).
14. R. H. Eich, R. P. Cuddy, H. Smulyan, R. H. Lyons, *Circulation* **34**, 299 (1966).
15. This work was supported by NIMH grant MH-13688 and research scientist award K5-6489 to S.M.S. We thank A. Crist for technical assistance.

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Tracheobronchial Epithelial Multinucleation in Malignant Disease

Abstract. *Multinucleated tracheobronchial ciliated epithelial cells seen in smears from 112 patients suffering from a wide variety of malignant tumors were found to be 2.08 times more numerous than in a control group comparable in sex, age (decades), and smoking habit but without prediagnosed malignancies. The recognition of this phenomenon may lead to the development of a new test for the diagnosis of occult cancer and may open new pathways for investigation of cancer-host relationships.*

In a series of ongoing studies we are conducting on the exfoliative cytology of the tracheobronchial tree of patients undergoing general endotracheal anesthesia for surgery, thus far totaling 2983 cases, it has become increasingly evident to us that the morphology of the ciliated epithelial cells was affected by a wide variety of stimuli (*1*). We also gained the impression that smears from patients suffering from known systemic malignancies contained unusually large numbers of multinucleated ciliated cells. We have, therefore, carried out a retrospective pilot study to ascertain the statistical significance of this phenomenon. The preliminary results of this effort have been sufficiently dramatic to warrant preliminary publication.

Smears known to have contained at least 200 ciliated cells from 112 patients suffering from malignant tumors were reviewed, and the percentage of noncancerous cells containing more than one nucleus was calculated in relation to the total number of ciliated cells seen in each smear. The maximum number of nuclei in any one cell per smear was also noted. A randomly selected control group of smears from patients without known malignancies, and matched exactly by decade of age, sex, and smoking habit (*2*), was retrieved from the files and similarly examined. No patient was included who had received chemotherapy with alkylating agents or immunosuppressive drugs, or treatment with ionizing radiations, for at least 2 years before the collection of the specimen, to avoid contamination of data by changes in tracheobronchial cytology due to the use of these agents (*3*). All smears had been made from secretions obtained by suction of the tracheal tube immediately after intubation. This material was spread on microscope slides, spray-fixed at once, and stained (Papanicolaou). Microscopic examination was carried out by two of us (J.C. and J.S.K.), each of whom was unaware of the origin of the smears which had been previously mixed and tagged by the other members of the study group. Mean scores of the readings of both observers were used for final analysis of data. The mean percentages of multinucleated cells and the standard errors of the means were then calculated for all malignant and control groups and for subgroups divided by age, sex, smoking habit, and site of origin of tumors. Student's *t*-test for uncorrelated series was used for statistical analysis. Statistical significance was selected at values of $P < .02$.

The mean percentages of multinucleated cells in smears from patients with malignant disease and for all controls (Table 1) were 3.93 ± 0.22 and 1.89 ± 0.11, respectively ($P < .001$). If cases were subdivided by age, sex, and smoking habit, the difference in percentage of multinucleated cells between tumor and control groups remained statistically significant ($P < .02$) in each group. The higher percentage of multinucleated cells in men with malignant tumors (4.24 ± 0.45) over those from women suffering from the disease (3.78 ± 0.27) was not significant, nor was the higher incidence of multinucleation seen in smears from very heavy smokers with malignant disease (4.96 ± 0.65) over the mean incidence of multinucleation in all other smoking groups (3.93 ± 0.24).

When mean percentages of multinucleation were studied by site of origin of tumors, all tumor groups containing at least nine cases (colon and

Table 1. Mean ($\bar{X}$) percentage of multinucleated cells in patients with tumors and in controls; S.E., standard error; N.S., not significant.

Group studied	Tumors			Controls		*P*
	N	$\bar{X}$	S.E.	$\bar{X}$	S.E.	
Total study	112	3.93	0.22	1.89	0.11	< .001
Males	36	4.24	.45	1.87	.19	< .01
Females	76	3.78	.27	1.90	.15	< .01
Age groups						
10 to 39	14	4.09	.63	2.01	.39	< .01
40 to 69	68	3.81	.27	1.86	.14	< .001
70 and over	30	4.11	.55	1.89	.24	< .01
Smoking habit						
Nonsmoker	62	3.88	.31	1.19	.15	< .01
Light smoker	10	3.86	.57	2.00	.41	< .02
Medium smoker	10	4.02	.83	1.87	.40	< .02
Heavy smoker	21	3.60	.60	1.79	.24	< .02
Very heavy smoker	9	4.96	.65	1.89	.62	< .01
Site of origin						
Colon and rectum	32	3.60	.35	2.06	.22	< .01
Breast	28	4.28	.45	1.95	.29	< .01
Female genital	18	3.95	.55	1.73	.25	< .01
Stomach	9	3.54	.59	1.75	.37	< .02
Miscellaneous	6	3.59	.49	1.25	.34	< .02
Other digestive	6	4.58	1.65	1.27	.12	N.S.
Urinary system	5	4.19	0.92	2.60	.27	N.S.
Lymphomas	4	3.62	1.66	1.33	.21	N.S.
Bronchogenic	4	4.47	1.66	2.5	1.20	N.S.