our knowledge been reported in concert in cells of any long-term cultured human line other than HeLa. (ii) That none of the cultures exhibited marker No. 1, found in HeLa S_3 and many other HeLa strains, indicated the likelihood of a clonal derivation of all T-1 cultures. (iii) Continuing chromosome evolution in isolated strains of T-1 is inferred from the presence in each cell of additional unique markers. No metaphase in any of the cultures revealed a Y chromosome.

The five cultures were tested by gel electrophoresis for eight gene-enzyme systems (*8*). The allozyme phenotype of the cultures was identical to that of HeLa cells for these enzymes and conforms to previously published results. The probability of a genotype at these eight loci in an individual cell line being identical to HeLa is .0017 (*8*).

Results of HLA typing revealed an identical phenotype in all cultures, namely, a positive reaction for HLA-A2 antigen and negative results for HLA-A1, A3, A9, A10, and HLA-B5, B7, B12, B17 antigens. These results conform to the uncommon phenotype of HeLa cells that are found when a sensitive absorption procedure is used (*9*).

HeLa cells were present in the laboratory of origin at the time T-1 was initiated (*3*). During its establishment, initial proliferation diminished after one passage and 28 days in culture. By the end of 2 months it was noted that one of the original six tubes planted showed islands of epithelial cells of normal appearance which 5 days later could be transferred and proliferated rapidly. It is likely that these were, in fact, contaminating HeLa cells, descendants of one or a few HeLa cells.

The many conclusions based on experimental results obtained with the use of T-1 cells must be reevaluated in view of the origin of the cells and depending on the nature of the experiments. If a human cell, whether normal or tumor, sufficed in the protocol, the conclusions drawn remain sound. However, if one intended to collect data on normal, presumably diploid cells derived from kidney tissue of an 8-year-old male, most likely of Caucasian origin, and had, instead, used adenocarcinoma-derived heteroploid cells from the uterine cervix of a 31-year-old black woman, this would not be the case. Meanwhile we continue to search for long-term cultivated human cells of normal kidney origin.

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References and Notes

1. P. S. Furcinitti and P. Todd, *Science* **206**, 475 (1979).
2. G. W. Barendsen, T. L. J. Beusker, A. J. Vergroesen, L. Budke, *Radiat. Res.* **13**, 841 (1960); M. R. Raju, M. Guanapurani, C. Richman, *Br. J. Radiol.* **45**, 178 (1972); S. Okada, *Radiation Biochemistry*, K. I. Altman, G. B. Gerber, S. Okada, Eds. (Academic Press, New York, 1970), vol. 1; P. Todd, J. P. Geraci, P. A. Furcinitti, R. M. Rossi, F. Mikage, R. B. Theus, C. B. Schroy, *Int. J. Radiat. Oncol. Biol. Phys.* **4**, 1015 (1978); E. A. Blakely, C. A. Tobias, T. C. H. Yang, K. C. Smith, J. T. Lyman, *Radiat. Res.* **80**, 122 (1979).
3. J. van der Veen and L. Bots, *Arch. Ges. Virusforsch.* **8**, 230 (1958).
4. P. S. Furcinitti and P. Todd, personal communication (1979).
5. J. van der Veen (per F. Heessen), Department of Medical Microbiology, Geert Grooteplein Zuid 24, Nijmegen, The Netherlands; G. W. Barendsen, REP-Institutes of the Organization for Health Research TNO, 151 Lange Kleiweg, Rijswijk (ZH), The Netherlands; P. Todd, Department of Biochemistry and Biophysics, Pennsylvania State University, University Park 16802; E. A. Blakely, Biology and Medicine Division, Lawrence Berkeley Laboratory, University of California, Berkeley 94720; M. R. Raju (per N. Tokita), University of California, Los Alamos Scientific Laboratory, Los Alamos, N.M. 87545.
6. W. A. Nelson-Rees and R. R. Flandermeyer, *Science* **191**, 96 (1976); W. A. Nelson-Rees, L. Hunter, G. J. Darlington, S. J. O'Brien, *Cytogenet. Cell Genet.*, in press.
7. S. Pathak, M. J. Siciliano, R. Cailleau, C. L. Wiseman, T. C. Hsu, *J. Natl. Cancer Inst.* **62**, 263 (1979).
8. S. J. O'Brien, G. Kleiner, R. Olson, J. E. Shannon, *Science* **195**, 1345 (1977); S. J. O'Brien, J. E. Shannon, M. H. Gail, *In Vitro* **16**, 119, (1979). O'Brien kindly determined the genotypes at the following eight gene-enzyme loci: phosphoglucomutase-1 and -3 were both 1; 6-phosphogluconate dehydrogenase was A; esterase-D, 1; glyoxalase, 2; peptidase-D, 1; adenosine deaminase, 1; and glucose-6-phosphate dehydrogenase, A.
9. The HLA phenotype determinations were kindly performed by S. Ferrone after the absorption procedure was completed [M. A. Pellegrino, S. Ferrone, A. Pellegrino, *Proc. Soc. Exp. Biol. Med.* **139**, 484 (1972); R. Glaser, R. Lenori, S. Ferrone, M. A. Pellegrino, G. de-Thé, *Cancer Res.* **37**, 2291 (1977); S. Ferrone, personal communication (1980)].
10. This work was supported by contract number Y01-CP8-0500 between the National Cancer Institute and the Office of Naval Research.

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Dopamine Auto- and Postsynaptic Receptors: Possible Interference by Gallamine

Skirboll *et al.* (*1*) have provided data suggesting that dopaminergic autoreceptors in the substantia nigra are much more sensitive to small doses of dopamine agonists than many of the postsynaptic dopaminergic receptors in the caudate nucleus. The investigators may have introduced an artifact in their study, however, by using gallamine to immobilize the experimental animals.

Gallamine, a synthetic tubocurarine analog, is widely used because of its well-known blocking action of acetylcholine at the muscle end-plate receptors. Less well known is that gallamine may have a central anticholinergic action as well. When systemically administered, gallamine passes from blood into the cerebrospinal fluid (*2*), and it also exerts a direct action on the central nervous system as demonstrated by electrophysiological studies (*3*).

Although the precise interrelationships of the cerebral cholinergic and dopaminergic systems are not known, the two systems appear to be mutually antagonistic at a behavioral (*4*) as well as at a biochemical level (*5*), and they occupy overlapping areas in the substantia nigra and some other regions of the central nervous system (*6*). Among several possibilities, it has been proposed that acetylcholine could act at these areas as a presynaptic modulator of dopaminergic neurons (*7*).

The studies of Skirboll *et al.* were performed on the pars compacta of the substantia nigra and on the caudate nucleus. Besides their high dopamine content, these two regions display prominent acetylcholinesterase activity, suggesting that they are important sites for cholinergic-dopaminergic interaction (*7*). The response of these regions to either the systemic or the iontophoretic application of dopamine agonists is likely to be affected by cholinergic modifications induced by gallamine or other agents.

Although the central actions of systemic gallamine have been largely ignored, its use in a study of central dopaminergic mechanisms throws the validity of the results into serious question.

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References

1. L. R. Skirboll, A. A. Grace, B. S. Bunney, *Science* **206**, 80 (1979.
2. P. S. R. K. Haranath, A. Krishnamurty, L. N. Rao, K. Seshagirirao, *Br. J. Pharmacol.* **48**, 640 (1973).
3. L. M. Halpern and R. G. Black, *Science* **155**, 1685 (1967); A. Galindo, K. Krnjević, S. Schwartz, *Exp. Brain Res.* **5**, 87 (1968); E. S. Boyd, D. A. Meritt, S. Aroesty, M. Celso. *Am. J. Physiol.* **216**, 542 (1969); E. S. Munson and I. H. Wagman, *Arch. Neurol.* **28**, 329 (1973).
4. T. Schallert, I. Q. Whishaw, V. D. Ramirez, P. Teitelbaum, *Science* **199**, 1461 (1978); M. H. Van Woert, in *Brain Acetylcholine and Neuropsychiatric Disease*, K. L. Davis and P. A. Berger, Eds. (Plenum, New York, 1979), pp. 373–393.
5. F. Javoy, Y. Agid, D. Bouvet, J. Glowinski, *Brain Res.* **68**, 253 (1974); M. Trabucchi, D. L. Cheney, G. Racagni, E. Costa, *ibid.* **85**, 130 (1975).
6. D. L. Cheney, H. F. LeFevre, G. Racagni, *Neuropharmacology* **14**, 801 (1975); L. L. Butcher, K. Talbot, L. Bilezikjian, *Proc. West. Pharmacol. Soc.* **18**, 256 (1975).
7. L. L. Butcher, *Life Sci.* **21**, 1207 (1977).

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The concern Garcia-Buñuel expresses regarding the possibility that our results may be confounded by interactions between the paralytic we used and the specific substances under study is important and addresses an issue that is often ignored in pharmacological experiments. However, the gallamine-dopamine interaction hypothesized by Garcia-Buñuel is extremely unlikely.

Garcia-Buñuel's hypothesis necessitates at least three assumptions: gallamine crosses the blood-brain barrier, gallamine affects dopaminergic systems, and gallamine affects dopamine receptor sensitivity. We address each assumption in order.

Because charged molecules cross lipid membranes with difficulty, substances containing one quaternary amino group usually do not cross the blood-brain barrier in pharmacologically significant amounts (*1*); gallamine contains three quaternary amine groups. Thus, radioactively labeled gallamine has been shown not to enter the central nervous system (CNS) (*2*). However, some of the papers referred to by Garcia-Buñuel do present evidence that small amounts of gallamine may enter the CNS, and this possibility must be considered. In our case, however, even the crossing of gallamine into the brain could not have confounded our results, as shown below.

The references given by Garcia-Buñuel for dopamine-acetylcholine interactions deal with muscarinic drugs, whereas gallamine is a nicotinic antagonist. The paper referenced by Garcia-Buñuel to argue for anatomical dopamine-acetylcholine overlap shows the substantia nigra to have one of the lowest concentrations of acetylcholine in the brain. Although acetylcholinesterase is found in substantia nigra, it is well recognized that it is not a reliable marker for acetylcholine input (*3*).

Even if gallamine did affect the nigrostriatal system directly, a direct action of gallamine at the dopamine receptor must be hypothesized in order to confound our results, because microiontophoresis allows one to study the direct effect of dopamine on neurons. To our knowledge, no evidence has been advanced relating an interaction of acetylcholine with dopamine receptor sensitivity.

In addition, we have found that the dose response curves for dopamine neuron activity in rats with respect to apomorphine and dopamine are the same whether the animals are anesthetized with chloral hydrate or paralyzed with gallamine. Intravenous administration of gallamine to a respirated rat anesthetized with chloral hydrate does not alter dopamine cell firing rate or pattern. Furthermore, cholinergic drugs (for example, scopolamine and physostigmine), which pass the blood-brain barrier more easily than gallamine, have no detectable effect on dopamine cell activity.

From these data we conclude that in our studies gallamine did not confound the results.

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References

1. W. H. Oldendorf, *Annu. Rev. Pharmacol.* **14**, 239 (1974).
2. G. Dal Santo, *Br. J. Anesthesiol.* **44**, 321 (1972).
3. A. Silver, *Int. Rev. Neurobiol.* **10**, 57 (1967); M. J. Kuhar, in *Biology of Cholinergic Function*, A. M. Goldberg and I. Hanin, Eds. (Raven, New York, 1976), p. 3.

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Prediction of Hypolimnetic Oxygen Deficits: Problems of Interpretation

The recent report of Cornett and Rigler (*1*) presented a model to predict the areal hypolimnetic oxygen deficit (AHOD). This model was accurate over the range of the 12 lakes investigated (the total amount of variation $R^2 = 0.75$). The regression equation developed was

$$\text{AHOD} = -277 + 0.5R_p + 5.0\,\overline{T}_H^{1.74} + 150\ln(\overline{Z}_H)$$

where R_p is the areal phosphorus retention (in milligrams per square meter per year) from Dillon and Rigler (*2*), $\overline{T}_H$ is the mean volume-weighted temperature of the hypolimnion (in degrees Celsius), and $\overline{Z}_H$ is the mean thickness of the hypolimnion (in meters). But, despite the accuracy of this model to predict AHOD, we feel there are some logical and computational flaws in the approach used to generate the model.

The logical flaw centers around the term R_p, which appears to have been applied outside the limits of its normal validity in the Cornett and Rigler model. Nevertheless, this is a common problem with retention models (*2*) and is not too serious here.

The computational flaws are somewhat more serious than the logical flaw and jeopardize the validity of the model. A basic assumption of all least-squares multiple regression analyses is that the independent variables are not correlated (*3*). Although often overlooked in many multiple regression analyses, this assumption was particularly violated in the AHOD model of Cornett and Rigler. The negative correlation between $\overline{T}_H^{1.74}$ and $\ln(\overline{Z}_H)$ is very high, -0.69. A consequence of this high correlation is that the order in which the variables are entered into the stepwise multiple regression analysis will dramatically affect the predictive equation. The temperature term $\overline{T}_H^{1.74}$ was not significant in the original analysis, but this term may have been significant if added to the stepwise analysis before the hypolimnion thickness term, $\ln(\overline{Z}_H)$. The high negative correlation between $\overline{T}_H^{1.74}$ and $\ln(\overline{Z}_H)$ suggests that, if temperature were added to the stepwise regression first, the hypolimnion term might be nonsignificant. Should this be the result, the major point of the Cornett and Rigler report would be negated, that is, that $\overline{Z}_H$ played an important and unexpected role in predicting AHOD. We suggest a more detailed analysis of the significance of $\overline{T}_H$ and $\overline{Z}_H$ be made before any limnological conclusion about cause and effect be drawn. In particular, the stepwise regression analysis approach should be avoided.

We applied the Cornett and Rigler model with data from the Great Lakes. The predicted AHOD for Lake Michigan is 504 mg of O_2 per square meter per day for a hypolimnion 70 m thick and a total phosphorus concentration of 8 mg/m^3 (*4*); for Lake Superior the predicted AHOD is 486 mg of O_2 per square meter per day for a hypolimnion 130 m thick and a total phosphorus concentration of 4 mg/m^3 (*5*). We found that these predictions, although possible, are too high for both Lake Michigan and Lake Superior and have never been observed. Our conclusion is that the model appears very sensitive to $\overline{Z}_H$ and yields high AHOD values for any lake with a hypolimnion over 50 m thick.

An additional but less important problem with the computational technique is the transformation of the $\overline{Z}_H$ and $\overline{T}_H$ variables. The transformed variables, although statistically correct (*6*), are difficult to understand in terms of meaningful units. The temperature variable, in particular, is hard to decipher when raised to a power of 1.74. In some cases, transformation could change the meaning of an independent variable in the predictive model; that is, is $\ln(\overline{Z}_H)$ a change

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