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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29 April 1980

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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from hypolimnion thickness to volume?

In summary, although Cornett and Rigler (*1*) have developed an accurate model to predict AHOD for the 12 lakes investigated, we believe that the techniques used to formulate that model require a considerable number of assumptions. Additional effort should be directed toward understanding why the model gives accurate predictions in spite of the various assumptions used. Until such an effort is made, restraint should be placed on the use of this model.

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20 November 1979; revised 23 January 1980

Stimulated by studies that have documented the impact of human activity on the rate of oxygen depletion in the hypolimnion of freshwater lakes, we developed a simple empirical model (*1*) to predict areal hypolimnetic oxygen deficits (AHOD). The method that we used to generate the model, the interpretation of the model, and its predictive ability are all criticized by Chang and Moll (*2*). Although we will reply to Chang and Moll's first two points, we believe that the empirical testing of hypotheses is the most important step in advancing a predictive science and consequently we present a detailed analysis of their criticism of the model's predictive ability.

Chang and Moll first suggested that the model is logically invalid because it equates the areal retention of phosphorus (R_p) with sedimentation. This is not correct. We hypothesized that the input of organic matter into the hypolimnion is responsible for oxygen depletion in lakes. However, we chose R_p because it is easily estimated from existing models and because we expect it to be statistically correlated with the input of organic matter.

Chang and Moll also criticized our use of temperature raised to the power 1.74. Over the range of hypolimnetic temperatures found in our study lakes (4° to 13.5°C) many investigators (*3*) have demonstrated that biological respiration rates increase nonlinearly with temperature. Our analysis was influenced by this work. We chose 1.74 because this exponent was used by Hargrave (*4*) in his empirical equation to predict sediment respiration from ambient temperature. We retained this exponent in the final model because the use of other powers did not improve the predictive ability of our model.

Table 1. Comparison of predicted and observed AHOD in the Great Lakes (*10*).

Lake	$\bar{Z}_H$	Predicted AHOD	Observed AHOD
Superior	134	520.8	615
Michigan	70	504	?
Huron	50	391	394
Ontario	71	630	768
Erie (central)	3.9	431	409
Erie (eastern)	14.7	529	833

The mean thickness of the hypolimnion ($\bar{Z}_H$) was transformed to a logarithmic scale, not because we originally expected a logarithmic relation but because more data of sufficient quality were available from shallow lakes than from deep lakes. Consequently, our original data set was strongly skewed toward shallow lakes. We transformed the data to normalize our distribution of lake types for the statistical analysis. However, more recent studies suggest that the effect of $\bar{Z}_H$ does seem to decrease with increasing $\bar{Z}_H$ (*5*).

Chang and Moll also criticized our model because two of the independent variables, $\bar{Z}_H$ and $\bar{T}_H$, were negatively correlated in the original data set. They suggested that this correlation could have caused the apparent effect of $\bar{Z}_H$ on AHOD. If this suggestion were correct, our interpretation of the multiple regression analysis would certainly be suspect, although the predictive value of the equation for AHOD would be unaffected.

The important question is whether the apparent effect of $\bar{Z}_H$ on AHOD is real. Evidence that $\bar{Z}_H$ is essential to any interpretation of the model is provided by removing $\bar{Z}_H$ from the regression. Using exactly the same data set that we applied to develop our predictive model (*1*), we ignored $\bar{Z}_H$ and calculated the statistical model to be

$$\text{AHOD} = 230 + 0.8\,R_p - 2.2\bar{T}_H^{1.74} \quad (1)$$

We rejected this model because it implies that lakes are different from all other biological systems in that their respiration rate decreases as ambient temperature increases. This observation, although not stated explicitly in (*1*), can be deduced from the correlation analysis we presented; R_p is not highly correlated with $\bar{Z}_H$ or $\bar{T}_H$, whereas these two variables are themselves strongly negatively correlated. We also showed that ln ($\bar{Z}_H$) is positively, and $\bar{T}_H^{1.74}$ is negatively, correlated with the dependent variable AHOD [table 1 of (*1*)]. Therefore, removing $\bar{Z}_H$will cause a negative partial regression coefficient for $\bar{T}_H$, as Eq. 1 illustrates. This is consistent with the original multiple regression analysis. In that analysis, the partial regression coefficient for ln($\bar{Z}_H$) was highly significant after the effects of $\bar{T}_H$ and R_p were considered. Only the R^2 values were given in a stepwise progression. The conclusion that $\bar{Z}_H$ exerts a strong influence upon AHOD values is not negated, as Chang and Moll (*2*) have suggested. Several other more recent analyses also support the hypothesis that deeper lakes with a thicker hypolimnion have higher AHOD values (*5*-*7*).

Although we tested the predictive ability of our model by comparing the values predicted by the model with the observed AHOD values in an independent data set consisting of small oligotrophic lakes (N = 22), we also requested additional testing. Chang and Moll's (*2*) most potentially serious objection is that they have completed the testing that we requested and found the model to be inappropriate in the Great Lakes. However, they presented no measured AHOD values. We have now conducted this test for most of the Great Lakes, using data collected from the literature (*7*-*9*). The results (Table 1) show the expectation and conclusion of Chang and Moll to be erroneous. Lake Superior's measured AHOD is higher, not lower, than that predicted by the regression model. We could find no adequate data for Lake Michigan with which to test Chang and Moll's expectation for that lake. However, for the other deep basins of the Great Lakes the model makes good predictions or underestimates measured AHOD values (Table 1). Perhaps our model underestimates (rather than overestimates) the influence of depth upon AHOD values? However, the high AHOD values predicted by the model are certainly justified when the predictions are compared with the observed values.

Although we believe that we have countered Chang and Moll's objections, we shall be delighted if our model is found to be invalid as a result of further measurements. However, empirical the-

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Science **209** (4457), 721-722.
DOI: 10.1126/science.209.4457.721-a