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 9. We adopted this procedure for an important reason. With multiple regression procedures, it is possible to combine a number of nonredundant variables in such a way that, over a short segment of time, the result can be made to match any curve with similar frequency components. However, unless two sets of variables are reliably related, in a statistically stationary sense and for relatively long periods of time, it is highly improbable that the use of regression coefficients computed from their values at one period in time would result in accurate prediction when used with subsequent measurements. It is for this reason that we used the procedures described in the text.
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 11. The distinction between cortical PT and non-PT cells is basically an anatomical one, and need not imply functional differences. The axons of many PT cells terminate within sensory relay nuclei [S. J. Jabbur and A. L. Towe, *J. Neurophysiol.* **24**, 499 (1961)] and thus play no direct role in initiating movement. On the other hand, many of the non-PT cells within the motor cortex are part of the extrapyramidal motor system and may play an important role in the control of movement.
 12. The addition of several highly intercorrelated spike trains will not produce predictions of significantly greater accuracy than those derived from the activity of a single unit, because each contains essentially the same information.
 13. Predictions based on the multiple unit data were not only more accurate than those derived from single spike trains, but more reliable as well. For example, over the 20 flexion-extension cycles mentioned previously, the mean correlation ($\bar{r}$) between the combined unit data and the force trace was higher than that for any single unit, and the coefficients for individual movements varied considerably less ($\bar{r} = 0.82$, range = 0.76 to 0.92, S.D. = 0.08).
 14. In this short report we deemed it preferable to base our illustrations on data from a single, representative set of cells, so that the types of discharge patterns underlying the predicted curves and the improvements in accuracy resulting from addition of these patterns, could be clearly illustrated. We have made more detailed analysis of the spike train data from this and other sets of simultaneously observed units (D. R. Humphrey and E. M. Schmidt, in preparation).
 15. It is probable that our experiments suffered from the limitations inherent in many micro-electrode studies, namely, the tendency for the majority of successful recordings to be obtained from larger cortical neurons, with relatively few from the smaller, but more numerous, cells.
 16. We thank K. Davis for technical assistance, W. Pearson for reproduction of the figures, and Drs. K. Frank and R. Wurtz for reading the manuscript.

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Fluorescent Labeling of Chromosomal DNA: Superiority of Quinacrine Mustard to Quinacrine

We have recently reported a new technique for the selective labeling of chromosomal DNA with fluorescent DNA-binding agents in plant and mammalian metaphase chromosomes (1-3). The sharply defined, reproducible fluorescent bands are presumably indicative of biochemically different regions along the chromosomes. Although a number of fluorochromes have been studied in this investigation, most of them acridine derivatives, fluorescent alkylating agents are without question clearly superior to fluorochromes without alkylating groups. The best fluorochrome we have found is quinacrine mustard (4).

We have now applied this technique to the fluorescent labeling of human metaphase chromosomes (5, 6), and we are currently investigating the identification of human metaphase and interphase chromosomes in normal and pathological states, including neoplasias. The method also promises to be useful in studying the fine structure of human chromosomes and in karyotyping them. Visual identification of the 3, Y, and one of the D group chromosomes (5) and fluorometric differentiation of many other chromosomes, such as those in the B and C groups, have been reported (6). More recent papers on the fluores-

cent labeling of human chromosomes deal with the Y chromosome in interphase (7), the visual and fluorometric identification of abnormal chromosome 5 in the *cri du chat* syndrome (8), and the positive fluorometric identification of all human chromosomes in the normal metaphase plate (9).

Using this technique, other investigators have confirmed our studies on metaphase (10) and interphase (11) chromosomes and have also studied meiotic chromosomes (12). Several of these authors used quinacrine dihydrochloride as the fluorochrome, apparently for the reason that it is more readily available than quinacrine mustard. Quinacrine can be employed successfully in this technique (2, 3). However, we should like to point out several advantages of quinacrine mustard in contrast to quinacrine for chromosome fluorescence studies: the fluorescent bands produced by quinacrine are less clear, less stable, and possibly less reproducible; in plant chromosomes the quantitative fluorescence ratio (fluorescence of heterochromatin to that of euchromatin) achieved by quinacrine is substantially less than that resulting from quinacrine mustard (2); and quinacrine fluorescence, but not quinacrine

mustard fluorescence, fades rapidly on continued irradiation of the chromosomes with ultraviolet light. These observations are all consistent with the evidence that quinacrine mustard, which can form covalent bonds, binds approximately 20 times more strongly to DNA than quinacrine does (3).

Although quinacrine mustard is not as stable as quinacrine, refrigerated aqueous or ethanolic stock solutions of quinacrine mustard dihydrochloride can be used reliably for at least 1 week. This minor disadvantage is more than offset by the qualitatively and quantitatively superior results obtained with quinacrine mustard, and by the possibility that since the optimum concentrations of quinacrine (5 to 10 $\mu\text{g}/\text{ml}$) compared with quinacrine mustard (50 $\mu\text{g}/\text{ml}$) are in the ratio of 100 to 200, problems of washing out excess quinacrine from the chromosome preparations may be encountered.

In summary, although quinacrine is a useful fluorochrome, we caution against the indiscriminate use of quinacrine in preference to quinacrine mustard for fluorescence studies of plant, animal, or human chromosomes.

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