were chosen, one which produced little if any effect per se on the mice (1 mg/kg) and one which made mice definitely "sleepy" and quiet (5 mg/kg). With both doses there was significant protection against amphetamine for grouped mice (see Table 1, *III*).

In all this work, it must be emphasized that arbitrary timing in drug administration and termination of experiments has been employed. One could conceivably miss a "protective" effect of short duration, and the superior performance of phenobarbital over pentobarbital might be explained on such a basis. On the other hand, pentobarbital deaths were seen on occasion within an hour after injection. Doses of pentobarbital larger than 60 mg/kg were not employed because of the toxicity of this barbiturate at such levels. No attempt was made to improve the performance of pentobarbital or promazine by using repeated doses of these drugs.

It thus appears that the "single" animals die with about the same frequency after administration of amphetamine whether they are "untreated" beforehand or "treated" with phenobarbital, pentobarbital, or chlorpromazine. On the other hand, phenobarbital, chlorpromazine, or reserpine (in appropriate doses) afford definite protection to grouped animals, and indeed transform the three-per-canister situation to what is, in essence (from the standpoint of mortality), a one-per-can situation.

LOUIS LASAGNA
WILLIAM P. MCCANN*
Division of Clinical Pharmacology, Department of Medicine, and
Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore, Maryland

References and Notes

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4. This work was supported in part by a grant from the U.S. Public Health Service, National Institutes of Health (B-865-C), and in part by a grant from Eli Lilly and Company.
5. The LD_{50} and standard error values were obtained by the Spearman-Kärber method (*6*). The usual formula for variance (q_i) which is given by Finney as $p_i\ q_i/n_i$ assumes the independence of the results on all animals at the i^{th} dose. In the experiments reported here, in which animals were grouped in canisters, it would appear likely that the death of one animal in a cannister alters the situation with regard to the effects of amphetamine on the remaining two mice. Certainly, in our experiments, the animals in a given canister did not invariably either all live or all die. Our data indicated that an assumption of no correlation within canisters was false, as was an assumption of complete correlation within canisters. Because of the size of the experiments, it was deemed unwise to get an unbiased estimate of the variance from the differences between canisters. Accordingly, rather than use either of these assumptions in estimating the variance, a conservative procedure was adopted. For doses of amphetamine in the range at which not all mice died and not all survived, the variance of the proportion surviving was estimated by $(\frac{1}{2})\ (\frac{1}{2})/k = \frac{1}{4}k$, where k is the number of canisters at the i^{th} level. A similar estimate of variance was applied to the highest dose of amphetamine at which all animals survived, and to the lowest dose of amphetamine at which all animals died. (For doses outside this range, the variance estimate was set equal to zero.) Because of the conservative nature of this variance term, the confidence limits on the LD_{50} values are probably somewhat broader than they deserve to be. A special debt of gratitude is owed to Paul Meier for his suggestions on how to deal with the statistical problems raised in these studies.
6. D. J. Finney, *Probit Analysis* (Cambridge Univ. Press, Cambridge, ed. 2, 1952).
7. Chlorpromazine was generously supplied by Smith, Kline and French Laboratories.
8. N. C. Moran and W. M. Butler, Jr. [*J. Pharmacol. Exptl. Therap.* 118, 328 (1956)] report the LD_{50} as 190 mg/kg; Paul Mattis of Smith, Kline and French Laboratories reports it to be 92 mg/kg (personal communication).
9. Promazine was generously supplied by Wyeth Laboratories.
10. Reserpine was generously supplied by Ciba Pharmaceutical Products, Inc.

* Squibb fellow in medicine.

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Some Factors Affecting Fluorescence Maxima

The recent widespread use of recording spectrophotofluorometers, such as the Farrand and Aminco-Bowman instruments, as well as the use of manual instruments like the modified Beckman model DU (*1*), has resulted in a number of fluorescence maxima being reported in the literature. A recent paper (*2*) has brought to my attention the fact that not all investigators distinguish between "apparent" and "true" fluorescence maxima. An apparent maximum is one that is observed on a curve recorded directly by the instrument, while a true maximum is one that is observed on a curve which has been corrected for the various factors affecting it. The purpose of this report is to suggest some of the factors that affect fluorescence maxima, together with techniques for their correction, and to propose the usage of the terms *true* and *apparent* fluorescence maxima. The factors discussed should not be confused with those that affect quantum yield or relative intensity of fluorescence, which are adequately treated in a number of standard textbooks (*3*).

One factor which affects the fluorescence maximum is the degree of overlap between the long wavelength portion of the absorption spectrum and the short wavelength portion of the fluorescence spectrum. This factor is significant for a large number of compounds. If the fluorescence maximum lies to the short-wavelength end of the fluorescence spectrum (as is the case with many dye molecules and aromatic hydrocarbons), a shift of this maximum will occur toward longer wavelengths as the result of unequal absorption at each wavelength. This phenomenon is concentration-dependent and decreases with dilution.

Another factor is the relationship between the recorder-pen response (recorder time-constant) and the speed of the wavelength drive of the analyzer monochrometer. If the former is too slow or the latter is too rapid, the recorder pen will not be able to register its maximum response at each increment, particularly if the fluorescence peak is sharp. With instruments in which the wavelength dial is driven by a multiple-speed motor, the importance of this phenomenon can be easily evaluated by comparing curves recorded at different drive speeds.

A factor that is often neglected is the variation of detector sensitivity with wavelength. Most detectors do not have linear response characteristics and thus are more sensitive to light of one wavelength than of another. If one is working in the region of the spectrum where the response curve of the detector has a large slope (such as the region beyond 470 mμ, when using an R.C.A. 1P28 photomultiplier tube), a considerable difference between the true and apparent maxima can occur. (In my laboratory I have found that a difference of 15 to 20 mμ is not uncommon.) This factor is inherent in every instrument, and compensation for it can be accomplished only by correction of the recorded spectrum, at each wavelength, for the response of the detector involved. This can be ac-

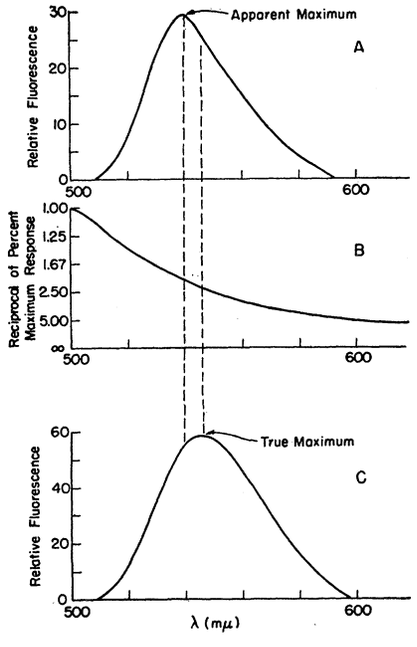

Fig. 1. Effect of detector response on recorded fluorescence curves. (*A*) Fluorescence spectrum as recorded; (*B*) detector response curve; (*C*) corrected fluorescence spectrum.

complished readily in the following manner. Figure 1*A* shows a fluorescence spectrum recorded with a detector that has the response characteristic shown in Fig. 1*B*. The true fluorescence spectrum (and hence true fluorescence maximum) is obtained by taking the product of 1*A* and 1*B* at each point and is illustrated by Fig. 1*C*. The true and apparent maxima are marked appropriately and differ by 7 mμ.

The naphthols provide an excellent illustration of this phenomenon. When a 1P28 photomultiplier tube is used, the visible fluorescence maximum of 1-naphthol, dissolved in concentrated sulfuric acid, is recorded at 528 mμ. The value obtained for the maximum, when corrected as described in the foregoing paragraph, is 544 mμ, a change of 16 mμ. Likewise, 1-naphthol dissolved in 0.2*N* sodium hydroxide shows an apparent maximum at 486 mμ, while the corrected maximum is at 500 mμ. Also, 2-naphthol dissolved in 0.2*N* sodium hydroxide and 0.1*N* sulfuric acid has apparent maxima of 424 mμ and 357 mμ, respectively, which, when corrected, give values of 429 mμ and 358 mμ. In general, response curves provided by the manufacturer of the phototube are sufficiently accurate, although for most precise results the detector may be calibrated by the National Bureau of Standards.

Scattered light from the source of excitation can also affect the fluorescence maximum and is dependent on the spectral band-pass of the analyzer monochrometer. If the fluorescence of the solution is weak, scattered light from the source can be reflected from the solution into the analyzer. If this light is of the appropriate wavelength (that is, if it is near the fluorescence maximum) it can cause a shift in the maximum recorded. The extent of this phenomenon can be readily checked by running blank solutions.

The accuracy of the wavelength calibration of the analyzer monochrometer is very important in locating the fluorescence maximum. The calibration can be checked with a low-pressure mercury arc or a hydrogen lamp (*4*).

Another factor is the slit width (spectral band-pass) of the analyzer monochrometer, for if this is too large it is conceivable that a false maximum might be obtained. This is especially true if the spectral band-pass changes with wavelength, as it does for prism instruments. The effect of this factor can be evaluated by running spectra at increasingly smaller slit widths and noting any shifts in the maximum.

A number of incidental factors can affect the fluorescence maximum. An impurity in the sample material or the solvent would have an effect if the impurity present were fluorescent. Many materials (such as benzene or alcohol) may contain traces of fluorescent impurities which could cause errors of this sort. It is often difficult to remove impurities of this type by conventional purification procedures. Fluorescence that results from the solvent can be detected by use of a blank.

A number of factors have been presented in this discussion which influence the value for the fluorescence maximum of a given compound, recorded on a specific instrument. If recorded curves have not been corrected, they are specific only for that particular instrument. However, they may be reproducible from laboratory to laboratory on an instrument of a given type, but this is not a sufficient criterion for a true maximum. It is therefore proposed that these uncorrected maxima be called *apparent* fluorescence maxima and that only those which are corrected for the factors discussed in this report be labeled *true* fluorescence maxima.

DAVID M. HERCULES
Department of Chemistry and
Laboratory for Nuclear Science,
Massachusetts Institute of Technology,
Cambridge

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Formation of Gibbsite Aggregates in Latosols Developed on Volcanic Ash

The segregation of gibbsite on dehydration into light-colored, irregular-shaped aggregates has been observed in certain Hawaiian soils which have developed on andesitic volcanic ash occurring in the rain forest areas. These soils have been classified by Cline *et al.* (*1*) into the hydrol humic latosol soil group. These soils have been developed by the intense and rapid weathering of andesitic volcanic ash under a warm climate having a heavy rainfall ranging from 120 to 350 inches with no season which can be considered dry. Under these conditions, the primary silicate minerals of the volcanic ash have decomposed, and many products of weathering have been leached from the soil. The free and rapid percolation of water has provided conditions favoring the removal of the released soluble silica. The desilication has been accelerated by the organic acids, which are provided by the decomposition of the mixed fern vegetation.

Table 1. Chemical composition of the light-colored aggregates of gibbsite formed on dehydration of soils of the hydrol humic latosol group. All figures are percentages.

Location	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	H_2O
Onomea	0.89	61.88	0.88	2.76	31.88
Kaiwiki	0.72	63.03	1.20	3.02	33.09
Hilo Sugar Co.	0.62	62.22	2.28	1.28	33.40
Hakalau	0.90	61.60	2.08	4.52	31.52
Pepeekeo	0.69	62.81	0.88	2.22	32.60
Hilo Forest Reserve	0.58	63.30	0.90	1.22	32.89
Average	0.72	62.46	1.37	2.50	32.55

The greatest development of the soils of the hydrol humic latosol group occurs on the beds of andesitic ash found on the slopes of Mauna Kea on the island of Hawaii. These soils are smeary, gelatinous clays which, upon dehydration, form a mixture of light- and dark-colored aggregates. The aggregates will not rehydrate to form the clay but instead become very water-stable aggregates. The volume weight of these soils is extremely low, ranging from 0.1 to 0.7 and averaging approximately 0.5. The soils often contain from 60 to 65 percent water; however, they have formed a very stable land surface.

The mineral and chemical composition of typical soils of the hydrol humic latosol group has been reported by Tamura *et al.* (*2*). The major mineral constituents which were identified and estimated in this report (*3*) are as follows: gibbsite, 25 to 33 percent; allophane, 13 to 26 percent; goethite, 10 to 34 percent; magnetite, 6 to 19 percent; and anatase, mica, silica, and quartz in minor amounts.

On drying, these soils lose volume. In the process of drying, light reddish-brown mottles are formed by congealing gel-like structures which separate from the darker-colored matrix. On further drying, the light-colored areas form solid aggregates which feel gritty in the dehydrating system. Finally, on complete dehydration, a mixture of light- and dark-colored aggregates is formed. The light-colored aggregates have been identified as gibbsite, the trihydrate of aluminum oxide, by chemical and differential thermal analysis. Tamura (*4*) has also confirmed this identification by x-ray diffraction procedures. The chemical composition of the six separations of light-colored aggregates is given in Table 1. The average alumina content of the aggregates is 62.46 percent, and the average water loss between 110° and 400°C is 32.55 percent. Only minor quantities of silica, iron oxide, and titanium oxide were found in these aggregates.