conducted in the hope that such an approach will provide further information on the specific nature of the reaction controlled by each of the genes (*4*).

PAUL L. PFAHLER
H. H. KRAMER
ROY L. WHISTLER
Departments of Agronomy and *Biochemistry,*
Purdue University, Lafayette, Indiana

References and Notes

1. T. J. Schoch and E. C. Maywald, *Anal. Chem.* 28, 382 (1956).
2. J. C. Cameron, *Genetics* 32, 459 (1947); H. H. Kramer, R. L. Whistler, E. G. Anderson, *Agron. J.* 48, 170 (1956).
3. G. M. Dunn, H. H. Kramer, R. L. Whistler, *Agron. J.* 45, 101 (1953).
4. This article is journal paper No. 1055 of the Purdue University Agricultural Experiment Station.

30 November 1956

Spectrophotofluorometric Studies of 5-Hydroxyindoles and Related Compounds

The 5-hydroxyindole compounds that are of current interest in psychopharmacology (*1*) and in carcinoid tumor diagnosis (*2*) can be detected best by methods utilizing scanning spectrophotofluorometric procedures (*3*). Problems in the quantification of such procedures are caused by a variety of factors that are known to affect fluorescence—namely, (i) wavelength of excitation (absorbed light); (ii) concentration of the fluorescent substance in solution; (iii) nature of the solvent; (iv) *p*H; (v) temperature; (vi) foreign substances (quenching effects); and (vii) instrumental structure (*4*). In view of this variety, it is of interest to present spectrophotofluorometric data on 5-hydroxyindoles and related compounds in simple systems that are devoid of complicating variables. Such data are useful in the development of quantitative assays for these compounds (*5*).

The compounds investigated in this study and their sources are listed in Table 1. All compounds except those noted were dissolved in distilled water by prolonged vigorous shaking without heat. Difficulties with solubility were generally not encountered with the concentrations used. Spectrophotofluorometric measurements were made on the Aminco-Bowman scanning spectrophotofluorometer calibrated and adjusted to an activation reading of 350 mμ and a fluorescence reading of 450 mμ for quinine at a concentration of 0.1 μg/ml dissolved in 0.1*N* H_2SO_4 (*6*).

Data for any given compound in Table 1 were obtained as follows. (i) Activation and fluorescence maxima were determined by the method described in the instruction manual for the Aminco-Bowman scanning spectrophotofluorometer (*7*). (ii) The widest range of concentrations optimal for measurement wherein these maxima were constant was found. This was an essential preliminary to the development of a standard calibration curve for the compound under test. (iii) Using the now established activation and fluorescence maxima for instrument settings, we determined the range of concentrations (within the afore-mentioned range) that gave a linear response curve as measured by fluorescence intensity. In this way, a standard calibration curve for each of the compounds listed in Table 1 was plotted, and its region of linearity was thereby revealed. This is essentially the procedure that was used in the calibration of the Aminco-Bowman scanning spectrophotofluorometer by quinine (*6*).

The data obtained are set forth in Table 1. Only the maximum activation and fluorescence peaks are presented. These agree well with the values of Bowman *et al.* (*3*). Scanned activation and fluorescence spectra for any given compound also showed subordinate peaks, some of which were not clearly defined. Among these were scatter and harmonic peaks that are not important for purposes of identification. However, activation spectra of the 5-hydroxyindoles and their parent analogs generally showed a definite subordinate peak at 220 ± 5 mμ, and corresponding fluorescent spectra showed a definite subordinate peak at 670 ± 5 mμ. Although minor decreases in *p*H occurred with increasing concentration, especially of acidic compounds, these variations did not affect activation and fluorescence maxima under the conditions of the test. This confirms the findings of others (*3*).

Calculations from data in Table 1 (using Planck's energy equation $E = hc/\lambda$ and the proper conversion units) reveal empirical relationships worthy of note. Thus, it can be shown that the 5-hydroxy derivatives of tryptophan, tryptamine, or indoleacetic acid absorb less energy for activation than the parent compound by an amount equal to approximately ¼ electron volt (≃ 5.75 kcal/mole); conversely, the activated 5-hydroxyindole compound emits more fluorescent energy than the parent compound by an amount equal to approximately ⅛ electron volt (≃ 2.88 kcal/mole). Such calculations may be useful for purposes of identification of compounds of this type.

Spectrophotofluorometric data were obtained for 5-hydroxytryptophan, serotonin, 5-hydroxyindoleacetic acid, their parent analogs, and compounds of re-

Table 1. Spectrophotofluorometric data on 5-hydroxyindoles and related compounds. Only the maximum activation and fluorescence peaks are presented in Table 1. A plot of points within the linear concentration range on the abscissa vs. the linear fluorescence intensity (*FI*) on the ordinate gives a straight line standard calibration curve. Fluorescence intensity (*FI*) equals galvanometer needle deflection (transmission scale) multiplied by meter multiplier readings on the Aminco photomultiplier microphotometer. Note that the insertion of a hydroxy group into position 5 of the indoles, tryptophan, tryptamine, and indoleacetic acid, increases the activation wavelength (*A*) by 15 mμ and decreases the corresponding fluorescence wavelength (*F*) by 15 mμ. Calculations based on this observation are discussed in the text. 1-Epinephrine bitartrate and 1-arternol bitartrate were made up in 5-percent acetic acid solution.

Compound	Wavelength maxima (mμ)		Concentration range (μg/ml)		Linear fluorescence intensity range (*FI*)
	Activation (*A*)	Fluorescence (*F*)	Constant maxima	Linear	
Indole*	280	350	0.01 → 10	0.01 → 1	0.015 → 1.47
Skatole*	290	370	0.001 → 10	0.001 → 1	0.005 → 1.36
1-Tryptophan*	280	355	0.001 → 10	0.1 → 7	0.075 → 1.65
5-Hydroxytryptophan†	295	340	0.001 → 10	0.001 → 5	0.015 → 5.20
Tryptamine-HCl*	280	355	0.001 → 10	0.005 → 7	0.008 → 5.3
Serotonin†	295	340	0.001 → 10	0.005 → 5	0.015 → 3.2
Indoleacetic acid*	285	360	0.001 → 10	0.001 → 3	0.015 → 3.8
5-Hydroxyindoleacetic acid‡	300	345	0.001 → 10	0.001 → 1	0.026 → 1.30
Lysergic acid diethylamide bitartrate§	320	430	0.001 → 10	0.005 → 7.0	0.013 → 15.0
1-Epinephrine bitartrate‖	280	320	0.18 → 18.2	0.18 → 18.2	0.023 → 0.87
1-Arternol bitartrate‖	280	320	0.02 → 20	0.2 → 20	0.022 → 0.96
Indoxyl acetate#	285	375	Measured only at 50 μg/ml		
Indoxyl**	310	395	Measured only at satd. soln.		
2,3-Dihydroxy indole**	315	400	Measured only at 5 μg/ml		
Oxindole**	300	345	Measured only at 5 μg/ml		
2-Methyl indole**	280	355	Measured only at 5 μg/ml		

* Eastman. † Nutritional Biochemicals. ‡ Upjohn. § Sandoz. ‖ Winthrop-Stearns. # Mann. ** Bios.

lated psychopharmacological interest by means of the Aminco-Bowman scanning spectrophotofluorometer. The instrument was calibrated to an activation reading of 350 mμ and a fluorescence reading of 450 mμ for quinine at a concentration of 0.1 μg/ml dissolved in 0.1*N* H_2SO_4. Data obtained were (i) activation and fluorescent maxima, (ii) the widest optimal range for measurement of concentrations in which these maxima were constant, and (iii) that range of concentrations (within the afore-mentioned range) that gave a linear response curve as measured by fluorescence intensity. Standard linear calibration curves for each of the compounds tested could then be plotted. Calculations revealed that the 5-hydroxyindoles absorb less energy for activation and emit more fluorescent energy in the activated state than the parent indole compounds.

HERBERT SPRINCE
GEORGE R. ROWLEY*
DOROTHY JAMESON
Veterans Administration Hospital, Coatesville, Pennsylvania, and *Department of Psychiatry, University of Pennsylvania, Philadelphia*

References and Notes

1. D. W. Woolley and E. Shaw, *Brit Med. J.* 1954 II, 122 (1954).
2. S. Udenfriend, H. Weissbach, A. Sjoerdsman, *Science* 123, 669 (1956).
3. R. L. Bowman, P. A. Caulfield, S. Udenfriend, *ibid.* 122, 32 (1955).
4. F. Wokes and E. J. Bowen, *Fluorescence of Solutions* (Longmans, New York, 1953); J. H. Harley and S. E. Wiberley, *Instrumental Analysis* (Wiley, New York, 1954).
5. This investigation was supported by research grant No. M-1015 (c) from the National Institute of Mental Health, National Institutes of Health, U.S. Public Health Service.
6. H. Sprince and G. R. Rowley, *Science* 125, 27 (1957).
7. Aminco-Bowman Scanning Spectrophotofluorometer Instruction Manual, pp. 11, 12.
* Present address: New Jersey Agricultural Experimental Station, Rutgers University, New Brunswick, N.J.

5 November 1956

Photoscanning Detection of Radioactive Tracers in vivo

Previously described scanning methods that provide a graphic representation of the distribution of radioactive materials within the human body have an arithmetic relationship between the gamma flux detected by the scintillation counter and the density of the resulting image (*1*). When the difference in radioactivity between the target and nontarget tissues is small, as in the case of brain-tumor localizations with iodinated human serum albumin, this type of equipment fails to delineate clearly the areas of abnormal concentration.

This preliminary report describes apparatus that was designed to give maximum contrast for a minimum difference in activity. This apparatus provides good definition with small dosages and an absence of background fogging without loss of significant data. It differs from the apparatus described by Kuhl *et al.* (*2*) in that even higher contrast can be obtained with equipment readily available in the average isotope laboratory, without the necessity for manufacturing a special electronic amplifier. The examples of clinical studies shown are representative of many such studies that have been performed with this instrument in the course of the past 18 months.

In this system, the signal from the scanning scintillation probe was fed into a single-channel pulse-height analyzer in which scattered radiation was discriminated against and by which the primary gamma emission was passed on as a signal to a count-rate meter. The output of the count-rate meter was then fed to a potentiometer-type recorder (*3*). A 20-ohm wire-wound potentiometer was mechanically coupled to the pen drive wheel of the recorder in such a fashion that maximum deflection of the recorder gave minimum resistance through the potentiometer. This potentiometer was in series with a small, tungsten-filament light source (*4*) that was mechanically fixed to the scanning probe so that the physical relationships of the two were constant. The electric supply for the tungsten filament was provided by a 6.3-v filament transformer. The light source was focused into a narrow slit by a 0.5-in. diameter Lucite rod acting as a cylindrical lens. Screen type x-ray film in an x-ray film cassette was placed beneath the light. The opaque face of the cassette was replaced by transparent red plastic 1/16 in. thick. The red plastic prevented fogging of the film from external light sources if the room lights were off, but allowed exposure of the film by the concentrated light source of the instrument. This obviated the need for a lightproof film holder.

As the probe passes over the patient's body and detects an increased gamma flux, the count-rate meter drives the recorder, which in turn removes resistance from the light circuit. In consequence, the light increases in brilliance and the film is exposed. Because of the marked dependence of light emission from a tungsten filament on changes in current, extremely high contrast is obtained, so that the net result can be a 95 percent increase in film density for a 10 percent increase in count rate.

Figure 1*A* is a photoscan of a normal thyroid with 4.5 μc of I^{131} in the gland; Fig. 1*B* is the image of a metastatic carcinoma of the thyroid with 10.3 μc in its substance, superimposed over an x-ray of the region involved; Fig. 1*C* is a lateral scan; Fig. 1*D* is an anteroposterior scan of a patient with a 2- by 1.5- by 1-cm

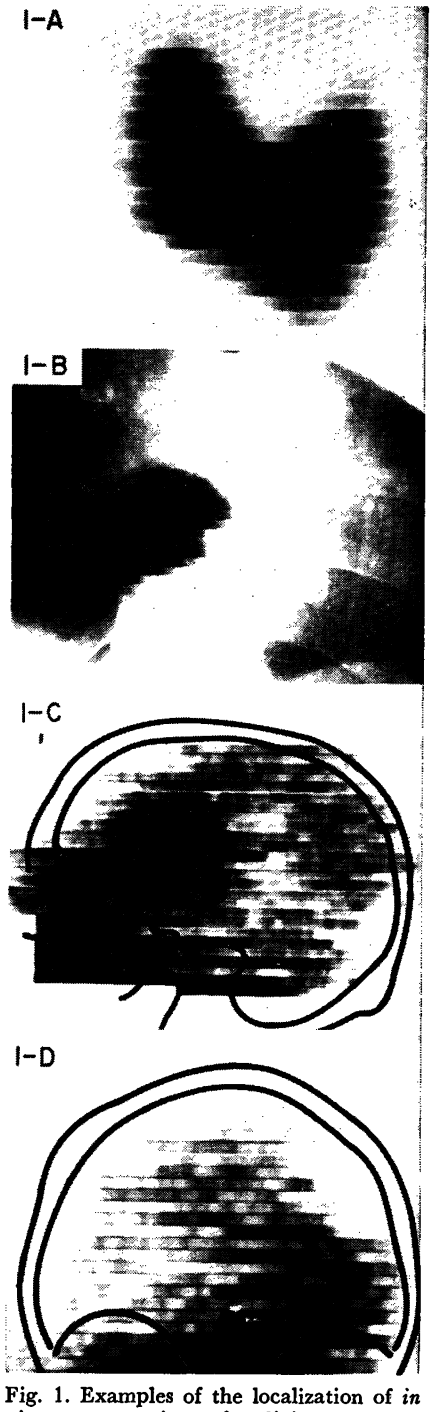

Fig. 1. Examples of the localization of *in vivo* concentrations of radioisotopes with photoscanning techniques.

cerebral metastasis to the left temporal lobe from a primary carcinoma of the breast. This patient received an intravenous administration of 300 μc of I^{131}-labeled human serum albumin 24 hours before the scan (*5*). It is important to note that the count rate over the site of the lesion was only 14 percent higher than the expected normal count rate for this position.

These studies were performed with a 1- by 1-in. sodium iodide (thallium activated) crystal having a lead collimator