REFERENCES

(1) Shealy, Y. F., Krauth, C. A., and Montgomery, J. A., *J. Org. Chem.*, **27**, 2150(1962).
(2) Shealy, Y. F., and Krauth, C. A., *J. Med. Chem.*, **9**, 34(1966).
(3) Shealy, Y. F., Montgomery, J. A., and Laster, W. R., Jr., *Biochem. Pharmacol.*, **11**, 674(1962).
(4) MacDonald, C., Wollner, N., Ghavimi, F., and Zweig, J., *Proc. Am. Assoc. Cancer Res.*, **8**, 43(1967).
(5) Loo, T. L., Stasswender, E. A., Jardine, J. H., and Frei, E., III, *ibid.*, **8**, 42(1967).
(6) Shealy, Y. F., Krauth, C. A., Pittillo, R. F., and Hunt, D. E., *J. Pharm. Sci.*, **56**, 147(1967).
(7) Shealy, Y. F., and O'Dell, C. A., *J. Med. Chem.*, **9**, 733(1966).
(8) Clarke, D. A., Barclay, R. K., Stock, C. C., and Rondestvedt, C. S., Jr., *Proc. Soc. Expll. Biol. Med.*, **90**, 484(1955).
(9) Rondestvedt, C. S., Jr., and Davis, J. D., *J. Org. Chem.*, **23**, 200(1957).
(10) Shealy, Y. F., and Krauth, C. A., *Nature*, **210**, 208(1966).
(11) Shealy, Y. F., Krauth, C. A., Holum, L. B., and Fitzgibbon, W. E., *J. Pharm. Sci.*, **57**, 83(1968).
(12) *Cancer Chemotherapy Rept.*, **No. 25**, 1 (1962); **No. 1**, 42 (1959).
(13) Laster, W. R., Jr., Schabel, F. M., Jr., Skipper, H. E., Wilcox, W. S., and Thomson, J. R., *Cancer Res.*, **21**, 895 (1961).
(14) Skipper, H. E., Wilcox, W. S., Schabel, F. M., Jr., Laster, W. R., Jr., and Mattil, L., *Cancer Chemotherapy Rept.*, **29**, 1(1963).
(15) Kline, I., Venditti, J. M., Tyrer, D. D., and Goldin, A., *Cancer Res.*, **26**, 853(1966), and references cited therein.
(16) Skipper, H. E., Schabel, F. M., Jr., and Wilcox, W. S., *Cancer Chemotherapy Rept.*, **51**, 125(1967).
(17) Shealy, Y. F., Struck, R. F., Holum, L. B., and Montgomery, J. A., *J. Org. Chem.*, **26**, 2396(1961).
(18) Loo, T. L., and Stasswender, E. A., *J. Pharm. Sci.*, **56**, 1016(1967).

Keyphrases

Imidazoles—synthesis
TLC—separation, identity
UV light—identity
Antitumor activity—imidazoles
Degradation, imidazoles—light exposure

Specificity of Fluorometry of 5-Hydroxytryptamine by Means of Products with Ninhydrin

By W. B. QUAY

The specificity and relevant technical factors of the fluorometry of 5-hydroxytryptamine (5-HT) by means of its product with ninhydrin (1,2,3-indantrione) were studied in experiments with 121 test compounds and tissues from rats and cockroaches. Aromatic amines producing fluorescence capable of confusion with that from 5-HT were: a number of carboline derivatives, a few hydroxyindole congenors of 5-HT, 4-(aminomethyl)-piperidine, and 2-(2-aminoethyl)-pyridine. Comparisons of results from two different fluorometric procedures showed close correspondence with most mammalian tissues, but not for stomach, nor for cockroach tissues. More significant in the discrimination between 5-HT and the related compounds were the time and temperature-dependent fluorescence development and decay curves of incubations with ninhydrin.

5-HYDROXYTRYPTAMINE (5-HT) occurs widely and is of special interest in its association with neural and neuroeffector mechanisms (1). Its measurements in tissues has been by both bioassay and chemical means. Of the latter, fluorometric procedures are of paramount utility. One of these depends upon the fluorescence of 5-HT (and other 5-hydroxy and 5-methoxy indoles) in strong acid (fluorescence maximum at 540 mμ, excitation maximum at 295 mμ) (2, 3). Another depends on the fluorescence of a reaction product of 5-HT and ninhydrin (1,2,3-indantrione hydrate) (4, 5). This second technique is the more sensitive and has been thought to be more specific (5). In practice this appears to be true for most mammalian and reptilian tissues studied so far (6). But for some groups of animals and kinds of tissues the results by the two methods are divergent, with the ninhydrin technique giving falsely high values (Quay, unpublished results).

This report is a summary of an evaluation of the specificity of the fluorometric technique for 5-HT with ninhydrin, with special attention to amines most apt to produce a reaction product, and to derivatives and relatives of 5-HT. Several types of limitations in specificity are described, and methods for checking specificity are noted.

EXPERIMENTAL

The 121 compounds tested consisted of 72 indoleamines and their derivatives, 11 catecholamines and

Received March 11, 1968, from the Department of Zoology, University of California, Berkeley, CA 94720
Accepted for publication June 14, 1968.
This work was supported in part by a research grant (NB-06296), U. S. Public Health Service, Bethesda, Md.

derivatives, and 38 amino acids and other N-containing compounds. Immediately before use small samples of these were weighed to the nearest microgram with a quartz helix balance (Microchemical Specialties Corp.), dissolved, then diluted to a standard concentration. These and all other solutions were made with demineralized distilled water.

The fluorescence of the compounds at 490 mμ with excitation at 380 mμ (uncorrected) was measured with an Aminco-Bowman spectrophotofluorometer after five different producers: (*a*) untreated and dissolved in 0.05 *M* pH 7.0 sodium phosphate buffer; (*b*) dissolved in 0.05 *M* pH 7.0 buffer and incubated with ninhydrin at either 60 or 75°, and in a few cases at 40 and 85°, for a precise time, followed by quick cooling and dilution (1 μmole in 250 μl. with 50 μl. 1.78% ninhydrin + 250 μl. H_2O); (*c*) dissolved in 0.1 *N* HCl (DuPont) 0.5% *l*-ascorbic acid (Eastman), washed with diethyl ether, extracted into *n*-butanol (Eastman), passed into 0.05 *M* pH 7.0 buffer, and reacted with ninhydrin at 60° for 1 hr. [method of Wilhoft and Quay (6)]; (*d*) the same procedure but without the ether washes; (*e*) a scaled-down version of the method of Snyder *et al.* (5) summarized as follows: (*a*) 1 ml. of sample, blank, or standard in 0.4 *N* perchloric acid is placed in a 5-ml. glass-stoppered centrifuge tube (for tissue samples, the supernatant of an ice-cold homogenate in 0.4 *N* perchloric acid is used); (*b*) the pH is brought to 10 by adding NaOH and checking with a glass electrode pH meter; (*c*) 100 μl. 0.5 *M* borate buffer at pH 10, 600 mg. NaCl (Merck reagent), and 3 ml. of *n*-butanol are added; (*d*) the tubes are stoppered, shaken, and centrifuged; (*e*) the butanol supernatant is transferred to a 15-ml. glass-stoppered centrifuge tube containing 250 μl. 0.05 *M* phosphate buffer at pH 7.0 and 8 ml. of heptane (Phillips Petroleum, pure); (*f*) this second tube is stoppered, shaken, centrifuged, and most of the heptane phase is aspirated off and discarded; (*g*) 50 μl. of 1.78% ninhydrin is added to the aqueous phase, which is then incubated for 30 min. in a water bath at 75°; (*h*) immediately 250 μl. H_2O is added, the tube is agitated, and cooled.

RESULTS

Fluorescence of Carbolines With and Without Ninhydrin—It was found that of the compounds studied, a number of the carbolines (Fig. 1) and their derivatives at pH 7.0 fluoresce intensely at 490 mμ when irradiated at 380–385 mμ, without prior incubation with ninhydrin. Carbolines and derivatives could be divided into three groups on the basis of this fluorescence (Table I): (*a*) those with strong, moderate, or weak fluorescence without ninhydrin and some reduction in fluorescence intensity after incubation with ninhydrin for 1 hr. at 60°; (*b*) those with weak fluorescence without ninhydrin and with increased fluorescence or little change after ninhydrin, and (*c*) those lacking notable fluorescence at 490 mμ with and without ninhydrin. In general, carbolines fluorescing most strongly were those with double bonds at positions 2–3 and 4–5 (Fig. 1). Derivatives (tetrahydro) lacking these double bonds include the four with no notable fluorescence at 490 mμ.

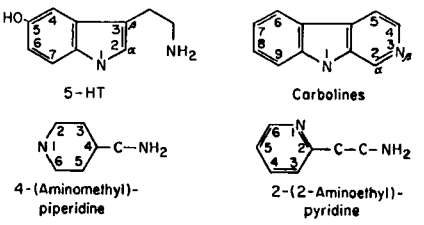

Fig. 1—Compounds giving fluorescence at 490 mμ (excitation at 380 mμ) either in neutral solution alone (carbolines) or after incubation with ninhydrin (others).

TABLE I—RELATIVE FLUORESCENCE OF CARBOLINES AND RELATED COMPOUNDS WITHOUT (0) AND WITH (+) PRIOR INCUBATION WITH NINHYDRIN[a]

Compd.	Relative Fluorescene 0	+
Group *A*		
Harmaline	>1,000	60
Harmalol	>1,000	10
Harmane	450	336
6-Methoxy-harmane	412	303
Harmine	403	278
2-Methylnorharman	318	258
2-Methylharmine	314	192
2-Methyltetrahydroharmol	301	13
2-Methyl-leptafluorine	153	0
6-Methoxy-*l*-tetrahydro-norharmanone	39	31
Tetrahydronorharmane carboxylic acid	36	19
Leptaflorine	25	1
6-Methoxy-tetrahydro-harman	15	5
N-(*py*)-propyltetrahydro-harmine	11	5
Eleagnine-1,3-dicarboxylic acid	10	1
Harminic acid	9	3
Noreleagnine	8	5
Tetrahydroharmane carboxylic acid	5	3
Leptacladine	3	0
Group *B*		
6-Methoxy-harmalan	5	32
6-Hydroxy-tetrahydro-harman	4	24
N-(*py*)-propyltetrahydro-harmane	3	6
Group *C*		
1-Oxo-1,2,3,4-tetrahydro-beta-carboline	0	0
Raunitidine	0	0
Tetrahydroharman	0	0
1-Tetrahydronorharmanone	0	0

[a] Compounds in pH 7.0, 0.05 phosphate buffer either incubated 1 hr. at 60° with ninhydrin (1 μ*M* in 250 μl. with 50 μl. 1.78% ninhydrin) or with H_2O alone, then diluted; fluorescence at 490 mμ; excitation at 380–385 mμ; tabulated values are means of 3 to 4 replicate pairs of tubes after subtraction of blanks (= incubated buffer with and without ninhydrin).

Carbolines with the above fluorescence characteristics were not significantly extracted by *n*-butanol in the three procedures studied, with four exceptions (Table II). The three extraction methods did not differ greatly in their results as far as these four carbolines are concerned. However, a slight reduction in the amount of 2-methylnorharman carried through the procedure was brought about

TABLE II—RELATIVE RECOVERY AND FLUORESCENCE PRODUCTION BY INDOLE DERIVATIVES ASSAYED ACCORDING TO THREE PROCEDURES[a]

Compd.[b]	Methods[c] I	II	III
5-HT	100	100	100
2-Methylnorharman	49	67	60
2-Methylharmine	40	32	38
2-Methyltetrahydroharmol	8	15	2
Harmalol	2	5	≃0
6-HT	7	4	—

[a] Comparisons made with 5-HT set at 100; results are means of two to four replicate series; fluorescence at 490 mμ; excitation at 380–385 mμ. [b] Tested at concentrations of 6–16 mμmoles/ml. [c] I = procedure of (6) with ether washes; II = the same, minus ether washes; III = procedure of (5).

by the ether washes, and possibly a slight reduction in the amounts of the hydroxylated compounds (2-methyltetrahydroharmol and harmalol) occurred in Method III, employing perchloric acid initially and a higher incubation temperature terminally.

Fluorescence of 5-HT and Other Indoles—In neutral 0.05 *M* phosphate buffer, 5-HT, and certain other indoles fluoresced at 490 mμ when irradiated at 380 mμ after having been incubated with ninhydrin. None fluoresced at 490 mμ without incubation with ninhydrin. The number of indoles in addition to 5-HT behaving this way was small, but depended in part on the time, temperature, and other conditions of the incubation with ninhydrin. Thus when equal concentrations (1×10^{-6} *M*/0.25 ml.) of these compounds in 0.05 *M* pH 7.0 buffer were incubated with ninhydrin at 75° simultaneously, the development and decay of the resultant fluorescent products followed different courses for the different compounds (Fig. 2). Fluorescence resulting during incubations of 30 min. or less could be largely attributed to 5-HT. With longer incubation times the fluorescence of products of some other normally occurring indoles increased as the 5-HT fluorescent product gradually decreased. The most intensely fluorescent of these other compounds was that from bufotenin (*N*-dimethyl-5-hydroxytryptamine; NDM-5-HT). Less, but still significantly, fluorescent were those from 5-hydroxyindole-3-acetic acid (5-HIAA) and

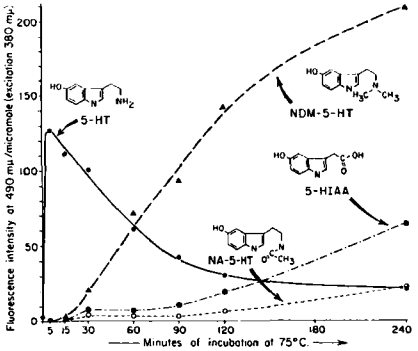

Fig. 2—Development and decay of fluorescent products during incubations of 5-hydroxyindoles with ninhydrin.

N-acetyl-5-hydroxytryptamine (NA-5-HT). Closely related and normally occurring indoles that failed to produce notable fluorescence at 490 mμ at any time during the incubation with ninhydrin at 75° included: tryptophan, 5-hydroxytryptophan, 5-methoxytryptamine, 5-methoxyindole-3-acetic acid, and melatonin (*N*-acetyl-5-methoxytryptamine). This was true if freshly dissolved indole standards were used. If older solutions were used, even after but a few days of refrigeration, some of the latter compounds through the agency of oxidation derivatives fluoresced with ninhydrin. Most significant among these was 5-hydroxytryptophan, several day-old solutions of which exhibited a pattern of fluorescence development intermediate to those of 5-HIAA and NDM-5-HT (Fig. 2).

A set of incubations identical to those represented in Fig. 2 but for the temperature being 60° instead of 75° showed the following differences: (*a*) Near peak fluorescence was attained by 5-HT with ninhydrin after an incubation of about 20 min. and constituted the start of a plateau in fluorescence intensity lasting through incubation times up to about 90 min., and with slow decline thereafter. (*b*) Fluorescence was produced neither by 5-HIAA nor by *N*-acetyl-5-hydroxytryptamine. (*c*) Fluorescence produced from bufotenine was not clearly evident until after an hour of incubation, and then its intensity was only 5% of that produced by 5-HT. Not until after 3 or 4 hr. of incubation did the fluorescence from bufotenine approach that from 5-HT.

Under these conditions besides 5-HT, 6-hydroxytryptamine (6-HT) also produced fluorescence with ninhydrin at 490 mμ with activation at 380 mμ. Although the resultant fluorescence per μmole of 6-HT was much less, 6-HT readily passes along with 5-HT through the extraction procedures (Table II).

Comparison was made of the fluorescence produced from the creatinine·H_2SO_4·H_2O complex of 5-HT with that from the hydrogen oxalate. On a per μmole basis the two were not significantly different and were at least within 5% of each other in level of fluorescence emitted.

Fluorescence of Other Amines and N-containing Compounds—The only other compounds that were both extracted by *n*-butanol and showed as much fluorescence as 5-HT after incubation with ninhydrin were 4-(aminomethyl)-piperidine and 2-(2-aminoethyl)-pyridine (Fig. 1). After certain conditions of incubation with ninhydrin a number of other compounds also produced fluorescence at 490 mμ (activation at 380 mμ), but of very low intensity. Furthermore, none of these amines passed the *n*-butanol extraction. Of these the most productive of fluorescence were lysine and cysteine (Table III).

Results from Tissues—Application of these methods to pooled samples of cockroach cephalic ganglia, nerve cords, and fat bodies demonstrated that the ninhydrin method (5, 6) gave mean "5-HT" values of 13, 10, and 22 mcg./g., respectively (Quay and Herman, unpublished), while the modified Bogdanski method (2, 3) gave trace values, comparable to those published by Welsh and Moorhead (7) for 5-HT in cockroach tissues (<0.02 to 0.05 mcg./g.).

Comparison of the results from the two methods

TABLE IV—ORGAN CONTENTS OF 5-HT ACCORDING TO TWO PROCEDURES

Organ[a]	N	5-HT[b] A: $\bar{x} \pm S_E$	5-HT[b] B: $\bar{x} \pm S_E$
Pineal	5	10.30 ± 1.04	11.27 ± 0.44
Duodenum	3	1.07 ± 0.08	1.06 ± 0.06
Stomach (pyloric)	4	0.77 ± 0.12	6.07 ± 0.41
Spleen	4	0.71 ± 0.24	1.27 ± 0.33
Lung	5	0.49 ± 0.16	0.45 ± 0.24
Liver	5	0.30 ± 0.02	0.22 ± 0.03
Adrenal	4	0.26 ± 0.04	0.25 ± 0.02

[a] Homogenates of adult rat organs provided equal aliquots for simultaneous determinations by two methods. [b] Mean ($\bar{x}$) mg/g. of 5-HT base [± standard error of the mean (S_E)] from N replicate organ samplings assayed terminally by: *A*—fluorescence at 540 mμ [excitation at 295 mμ; method of Quay (3)], *B*—fluorescence at 490 mμ (excitation at 380 mμ) after 1 hr. with ninhydrin at 60° [method of Wilhoft and Quay (6)].

applied to mammalian tissues showed equivalence for most but not all tissues tested (Table IV). Most clearly in the case of the pyloric stomach are the 5-HT values from the ninhydrin technique consistently and markedly greater—approximately eight times greater. The divergence of the spleen's values (Table IV) is not statistically significant, perhaps due to the great individual variation.

Study of the 5-HT content of different brain regions from rats by modified ninhydrin (6) and Bogdanski methods (2, 3) showed close agreement for most regions. But if higher temperatures and longer incubation times were used in the ninhydrin method, falsely high values for 5-HT were obtained. 5-HT either alone or added to brain homogenates had a fluorescent product with ninhydrin which followed the same time course of fluorescence during incubation, with rise, plateau and fall. However, from within homogenates of some brain regions ninhydrin-reactive, fluorescence-producing material was progressively more active at higher temperatures and longer incubation times. Identity of these other brain amines is being investigated.

DISCUSSION AND CONCLUSIONS

The specificity of fluorometric procedures for 5-HT based on a ninhydrin-reaction product is seen to be questionable in the absence of confirmatory results from other methods, and is less than appreciated previously (5). This is likely to be especially true for the tissues and body fluids of various invertebrate animals, which frequently have higher levels of free amines of diverse kinds (8). The same should be at least of potential concern in human and other mammalian tissues from pathologic and experimental situations in which free amines or metabolites may be abnormally high.

Auxiliary methods for the confirmation of the identity and apparent levels of 5-HT are numerous and may be found with the aid of recent reviews (9–11). Certain features of the ninhydrin reaction itself lend themselves also as means for ascertaining the identity of fluorescence-producing tissue constituents. The behavior of these according to temperature, time, and chemical environment will prove instructive in study of certain tissue amines, and will aid in confirming the identification of such

TABLE III—RELATIVE FLUORESCENCE OF INDOLES AND OTHER COMPOUNDS AFTER INCUBATION WITH NINHYDRIN FOR 1 hr. AT 60°[a]

Compd.[b]	Relative Fluorescence
5-Hydroxytryptamine	100
Bufotenine	5
6-Hydroxytryptamine	5
2-(2-Aminoethyl)-pyridine	79
4-(Aminomethyl)-piperidine	29
Lysine	9
4-Aminomethylpyridine	5
Cysteine	2

[a] Procedure and notes as in Table I. [b] The following indoles and derivatives failed to form products fluorescing at 490 mμ after incubation with ninhydrin for 1 hr. at 60°: *N*-acetyl-5-hydroxytryptamine, 5-methoxyindole-3-acetic acid, melatonin, 5-methoxytryptamine, 5-hydroxytryptophan, tryptophan, 5-hydroxyindole-3-acetic acid, bufotenine, tryptamine, α-methyltryptamine, 4-hydroxytryptamine, 3-indolylacetamide, 5-methyltryptophan, 3-methylindole, 3-(dimethylaminomethyl)indole (=gramine), *o*-phosphoryl-4-hydroxy-*N*-dimethyltryptamine (=psilocybin), 4-hydroxy-*N*-dimethyltryptamine (=psilocin), 4-hydroxytryptophan, 6-methyl-tryptophan, tryptophol, *N,N*-dimethyltryptamine, 5-benzyloxygramine, 3-(2-aminobutyl)-6-methoxyindole acetate, 6-methoxyacetyltryptamine, 5-methoxy-gramine, 6-hydroxy-5-methoxytryptamine, 6-hydroxymelatonin, *N*-acetyl-5-hydroxy-6-methoxytryptamine, 7-methyltryptamine, 5-methyltryptamine, kynurenine, dehydrobufotenine, *N*-methyltryptamine, 3-(2-dibutylaminoethyl)-5-hydroxyindole, 5-hydroxy-α methyltryptophan, 5-methoxytryptophan, 5-hydroxy-*N*ω-methyltryptamine, 5-benzyloxytryptamine, carbazole, and 5-aminoindole. Other amines and *N*-containing compounds studied and which produced extremely little or no fluorescence after 1 hr. with ninhydrin at 60° were: tyramine, 3,4-dihydroxyphenylalanine, dopamine, norepinephrine, epinephrine, phenylalanine, tyrosine, normetanephrine, octopamine, 3-methoxy-4-hydroxy-phenylethylamine, 3-methoxy-4-methoxy-phenylethylamine, histamine, 1,4-methylhistamine, 4-amino-6-hydroxypyrazolo(3,4-*d*)pyramidine, glycine, DL-α-alanine, serine, cystine, gamma amino-butyric acid, *p*-aminohippuric acid, threonine (allo-free), valine, norvaline, methionine, leucine, isoleucine, norleucine, citrulline, thyroxin, proline, aspartic acid, glutamic acid, arginine, histidine, ethionine, asparagine, adenine, adenosine, ethyl *p*-aminobenzoate, benzidine, 2,6-lutidine, glutamine, and pyridine.

constituents as 5-HT. Thus kinetic rather than endpoint analyses may be expected to be suitably informative.

In the ninhydrin methods, at least three classes of aromatic amines have been shown in these studies to be able to contribute fluorescence readily confused with that from 5-HT. These are: (*a*) carboline derivatives, fluorescent at 490 mμ without the necessity of reaction with ninhydrin; (*b*) certain hydroxyindoles resembling 5-HT and reacting with ninhydrin to produce similar fluorescence, and (*c*) certain other compounds having suitably reactive and exposed amine groups, such as those in 4-(aminomethyl)-piperidine and 2-(2-aminoethyl)-pyridine. At least many of these are not yet known to occur in animal tissues, and may be of theoretical interest only. On the other hand, hydroxyindole congeners of 5-HT do pose potential problems in determinations of 5-HT in tissues. Most of these can be excluded from 5-HT extracts by the use of suitable solvents and conditions in washing and extraction (3). Three may remain in part, however, to contribute something less than 5% of the fluorescence produced by the same amount of 5-HT. These are 6-HT (Table I), *N*-acetylserotonin and 5-hydroxytryptophan (5-HTP). The last two of these can be removed by suitable washes, such as with diethyl ether (3), a procedure not without other hazards however. 5-HTP is a problem as a fluorescence producer with ninhydrin only after some deterioration in solution has occurred.

The time course of fluorescent product formation from ninhydrin is different for these compounds as compared with 5-HT. Therefore, careful study can discriminate between them.

REFERENCES

(1) Quay, W. B., *Adv. Pharmacol.*, **6**, 283(1968).
(2) Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S., *J. Pharmacol.*, **117**, 82(1956).
(3) Quay, W. B., *Anal. Biochem.*, **5**, 51(1963).
(4) Vanable, J. W., Jr., *ibid.*, **6**, 393(1963).
(5) Snyder, S. H., Axelrod, J., and Zweig, M., *Biochem. Pharmacol.*, **14**, 831(1965).
(6) Wilhoft, D. C., and Quay, W. B., *Comp. Biochem. Physiol.*, **15**, 325 (1965).
(7) Welsh, J. H., and Moorhead, M., *J. Neurochem.*, **6**, 146 (1960).
(8) Holden, J. T., "Amino Acid Pools, Distribution, Formation and Function of Free Amino Acids," Elsevier, Amsterdam, The Netherlands, 1962.
(9) Sandler, M., "The Clinical Chemistry of Monoamines," Varley, H., and Gowenlock, A. H., Eds., Elsevier, Amsterdam, The Netherlands, 1963, p. 116.
(10) Hanson, A., "Handbuch der experimentellen Pharmakologie," vol. 19, Erspamer, V., Subed., Springer-Verlag, Berlin, Germany, 1966, p. 66.
(11) Erspamer, V., *ibid.*, p. 113.

Keyphrases

5-Hydroxytryptamine in tissue—analysis
Tissue analysis—5-hydroxytryptamine
Fluorometry, specificity—5-hydroxytryptamine analysis
Ninhydrin reagent
Amine interference—5-hydroxytryptamine analysis

Some Conditions Under Which Pentobarbital Stimulates Spontaneous Motor Activity of Mice

By NATHAN WATZMAN, HERBERT BARRY, III, WILLIAM J. KINNARD, JR., and JOSEPH P. BUCKLEY

The effects of 4 doses of sodium pentobarbital (10, 20, 40, 80 mg./kg., p.o.) on spontaneous motor activity of mice were tested in photocell cages during a 2-hr. period immediately after drug or saline administration. A total of 480 albino mice was divided among several experimental conditions. Increasing doses produced progressively increasing activity in the last 0.5 hr. when normal activity was at a low level. In the first 0.5 hr., the usual intense activity was unchanged by the three lower doses and greatly depressed by the highest dose. Throughout the session, the three lower doses had a stimulant effect on animals tested in a lighted environment in groups of five but not singly, and a small but consistent stimulant effect on both single and grouped animals in a dark environment. Animals fasted for 24 hr. prior to the test were greatly stimulated by the lowest dose and were greatly depressed by the highest dose. The results indicate that the stimulant effect of barbiturates requires both inhibitory influences on normal activity and conditions increasing susceptibility to arousal.

CLINICALLY, subhypnotic doses of barbiturates have been known to produce excitement, euphoria, and confusion rather than central nervous system depression (1). Many investigators have reported a stimulant effect in laboratory animals with low doses and depression with high doses. Kinnard and Carr (2) reported that spontaneous motor activity of mice was increased by low doses and decreased by high doses of sodium amobarbital and sodium secobarbital. A stimulant effect of low doses of barbiturates on spontaneous motor activity of mice has also been demonstrated in several subsequent experiments (3–7).

The stimulant action of barbiturates has been analyzed in situations where the drug enhances a previously rewarded response that was subsequently inhibited by punishment or nonreward (8). This stimulant effect has generally been attributed to a disinhibitory action which at certain doses is stronger than the intrinsic depressant action (9). Dews (10) reported that in a variety of food-rewarded tasks, conditions

Received May 2, 1968, from the Department of Pharmacology, University of Pittsburgh School of Pharmacy, Pittsburgh, PA 15213
Accepted for publication June 17, 1968.
Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.
This investigation was supported by grant MH-06540 from the National Institute of Mental Health, U. S. Public Health Service, Bethesda, Md. Preparation of the paper was supported by grant MH-13595 and by Research Scientist Development Award No. K03-MH-05921 (to Dr. Barry) from the same source.
The statistical analysis was done at the University of Pittsburgh Computer Center, supported by grant G-11309 from the National Science Foundation.