Biochemical Pharmacology, 1962, Vol. 11, pp. 617–625. Pergamon Press Ltd., Printed in Great Britain.

# THE EFFECT OF PYRIDINE ALDOXIME DODECYLIODIDE ON MONAMINE OXIDASE AND BOUND AMINES

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(*Received* 11 *February* 1962; *accepted* 20 *March* 1962)

**Abstract**—Pyridine aldoxime dodecyliodide (PAD) inhibits the monoamine oxidase (MAO) of rat liver and brain *in vitro* but not *in vivo*. Other long-chain quaternary compounds are also effective inhibitors *in vitro*. No preincubation is necessary, and the inhibition is irreversible. PAD is also an effective inhibitor of "solubilized" MAO. PAD lowers the 5-hydroxytryptamine (5-HT) levels in rat brain but has no effect on the catecholamine levels. It does not inhibit 5-hydroxytryptophan decarboxylase of rat kidney but does release 5-HT from mast cells *in vitro*. These findings suggest that PAD exerts a selective releasing action on bound 5-HT. Animals given sufficient PAD to deplete their 5-HT stores do not have the appearance of animals treated with reserpine. In addition, animals pretreated with phenylisopropylhydrazine (PIH), an inhibitor of MAO, and then given PAD do not have the appearance of animals pretreated with PIH and then given reserpine. Decamethonium and hexamethonium are also weak inhibitors of MAO *in vitro*.

PYRIDINE ALDOXIME dodecyliodide (PAD) was synthesized[1] to obtain a more lipid-soluble analog of pyridine aldoxime methiodide (PAM),[2] both of which are capable of reactivating cholinesterase inhibited by organophosphorous compounds such as diisopropylfluorophosphate. It was reasoned that the long-chain N-alkyl group would enhance the lipid-solubility of the quaternary pyridinium molecule and thus allow it to penetrate the blood-brain barrier, which is known to be most permeable to compounds of high lipid solubility.[3] PAM itself has been shown to penetrate into the brain,[4] although no similar direct evidence is available that PAD can pass the blood-brain barrier. Recent work has shown that PAD can inhibit cholinesterase, block conduction at the node of Ranvier, increase the permeability of nerve fibers to sodium ions,[5] and depolarize resting nerve fibers.[6] Some of these effects have been attributed to the reaction of PAD with the postulated acetylcholine receptor protein along the nerve axon.[5]

The work presented here shows that, in addition to these effects on acetylcholine metabolism and on the proteins associated with it, PAD exerts two important effects on the metabolism of serotonin (5-hydroxytryptamine, 5-HT): (i) inhibition *in vitro* of monoamine oxidase (MAO), the enzyme that is mainly responsible for the catabolism of 5-HT;[7] and (ii) release of 5-HT from brain and mast cells in which 5-HT is bound to particulate components.

## MATERIALS AND METHODS

5-HT and 5-hydroxytryptophan (5-HTP) were obtained from the California Corporation for Biochemical Research. Kynuramine was obtained from the Regis Chemical

Company; decamethonium, hexamethonium, PAM, and cetyl pyridinium iodide were purchased from the K & K Chemical Company. Stigmonene bromide was a gift of the Warner-Chilcott Company. Iproniazid phosphate was obtained from Hoffmann-LaRoche, Inc. Catron (JB-516) was obtained from Lakeside Laboratories. Harmaline was a gift of Professor Nicholas Giarman of this department. Thiamine and thiamine pyrophosphate were purchased from Schwartz Biochemicals, as was diphosphopyridine nucleotide. Succinylcholine, *d*-tubocurarine, neostigmene and N-methyl nicotinamide were obtained from various commercial sources. PAD was synthesized by the method of Wilson and Ginsburg.[1] Long-chain alkyl pyridinium compounds were synthesized by the method of Knight and Shaw.[8]

5-HT was determined spectrophotofluorometrically by the method of Udenfriend *et al.*[9] Numerous experiments were performed to show that PAD does not interfere with the determination of 5-HT by this method. Catecholamines were determined by spectrophotofluorometer according to a method of Bertler *et al.*[10] MAO activity was assayed spectrophotometrically.[11] All incubations were for 10 min within the housing of a Beckman DU spectrophotometer. The results obtained were occasionally checked by another method (Cotzias and Greenough[12]). 5-HTP decarboxylase was determined by the technique of Gaddum and Giarman.[13] Mitochondria were prepared from rat liver according to the method of Hogeboom,[14] and frozen at −20 °C until needed. Each milliliter of the mitochondrial suspension used contained the mitochondria from 1 g of liver (wet wt). MAO from such mitochondrial preparations was "solubilized" by the method of Zeller *et al.*[15] All compounds were administered to animals intraperitoneally. The rats used were males of approximately 150-g weight (Sprague-Dawley strain). The mice were DBA/2 males weighing about 20 g. The mast cells used were from the P815Y murine mastocytoma, ascites form.[16] PAD was dissolved in a solution containing $NaHCO_3$, 1 mg/ml. In all experiments involving PAD, the control animals were given the $NaHCO_3$ solution.

TABLE 1. EFFECT OF PAD ON THE MAO ACTIVITY OF LIVER AND BRAIN HOMOGENATES OF RAT, RABBIT, GUINEA PIG, AND MOUSE

| | % Inhibition of MAO | | | |
|---|---|---|---|---|
| | Liver | | Brain | |
| Species | $10^{-4}$ M* | $10^{-5}$ M | $10^{-4}$ M | $10^{-5}$ M |
| Rat | 90 | 25 | 41 | 16 |
| Rabbit | 69 | 20 | 60 | 20 |
| Guinea Pig | 100 | 20 | 40 | 12 |
| Mouse | 66 | 18 | 45 | 15 |

The brains and livers of adult animals killed by decapitation were excised, homogenized in 4 vol of cold distilled water, and filtered through cheesecloth; 0·2 ml of brain homogenate and 0·1 ml of liver homogenate were used for each assay of MAO activity. All assays were done in duplicate with less than 5% variation.

* Concentrations of PAD.

## EXPERIMENTAL

(1) *Effect of PAD on the MAO activity of homogenates of liver and brain of various species.* Table 1 demonstrates that PAD can inhibit the MAO of both liver and brain of a number of species. Because of the crudeness of these preparations it is not possible to say whether MAO of brain or liver is more susceptible to inhibition by PAD.

(2) *Effect of PAD on MAO activity of washed rat liver mitochondria and comparison of PAD with other inhibitors of MAO.* Table 2 shows that the inhibition of MAO *in vitro*, obtainable with PAD, is substantial. As can be seen in Table 3, PAD is less potent than is JB-516 as an inhibitor of MAO *in vitro*, but is more potent than iproniazid. Table 3 also indicates that a number of long-chain N-alkyl quaternary pyridine compounds can inhibit MAO *in vitro*.

TABLE 2. EFFECT OF PAD ON MAO ACTIVITY OF WASHED RAT LIVER MITOCHONDRIA *in vitro*

| Concentration of PAD | % Inhibition of MAO activity |
|---|---|
| $10^{-4}$ M | 100 |
| $5 \times 10^{-5}$ M | 75 |
| $10^{-5}$ M | 40 |
| $4 \times 10^{-6}$ M | 20 |

Preincubation of enzyme and PAD was not performed. Four determinations at each concentration of inhibitor were performed with less than 5% variation.

TABLE 3. COMPARISON OF PAD AND RELATED COMPOUNDS WITH PHENYLISOPROPYLHYDRAZINE AND IPRONIAZID AS MAO INHIBITORS *in vitro*

| Inhibitor ($10^{-5}$ M) | % Inhibition |
|---|---|
| JB-516 | 100 |
| PAD | 40 |
| Isonicotinic acid dodecyliodide | 40 |
| Dodecyl pyridine iodide | 25 |
| Tetradecyl pyridine iodide | 20 |
| Cetyl pyridine iodide | 20 |
| Iproniazid | 5 |

JB-516 and iproniazid were preincubated with the washed rat liver mitochondria for 15 min before kynuramine was added. Four assays with each inhibitor were performed with less than 5% variation.

TABLE 4. EFFECT OF PREINCUBATION ON THE INHIBITION OF RAT LIVER MITOCHONDRIA BY PAD

| Concentration of PAD | MAO activity (Δ O.D./10 min) No preinc. | Preinc. |
|---|---|---|
| | 0·172 | 0·170 |
| $4 \times 10^{-5}$ M | 0·100 | 0·100 |

The substrate, kynuramine, was added to the reaction mixture prior to the addition of MAO, or 30 min after MAO. In both series, reactions were run in duplicate with and without PAD.

(3) *Effect of preincubation on the inhibition of rat liver mitochondrial MAO by PAD.* PAD, unlike hydrazine-derived inhibitors of MAO, does not require preincubation to exert its maximal inhibitory action on MAO. Table 4 gives the results of an experiment showing that preincubation of MAO with PAD produced no effect on the inhibition obtained.

(4) *Effect of dialysis on the inhibition of rat liver mitochondrial MAO by PAD.* The hydrazine inhibitors irreversibly inhibit MAO, while harmaline is a reversible inhibitor.[17] The inhibition due to PAD is not reversible by extensive dialysis, despite the fact that no preincubation is necessary. The results given in Table 5 show that, in an experiment comparing harmaline and PAD, only the harmaline inhibition of MAO was reversible.

TABLE 5. EFFECT OF DIALYSIS ON PAD AND HARMALINE INHIBITION OF RAT LIVER MITOCHONDRIAL MAO

| Inhibitor | Δ O.D./10 min/1 ml of mixture | |
|---|---|---|
| | No dialysis | Dialysis |
| | 0·130 | 0·110 |
| PAD ($1·5 \times 10^{-3}$ M) | 0·0 | 0·0 |
| Harmaline ($1·5 \times 10^{-3}$ M) | 0·0* | 0·60 |

Of the mitochondrial suspension, 0·4 ml was added to 7·6 ml of a solution of $NaHCO_3$, 1 mg/ml. The same amount of mitochondria also was added to 7·6 ml of the $NaHCO_3$ solution which contained PAD at a concentration of $1·5 \times 10^{-3}$ M. Each mixture was divided in half. One-half was dialyzed for 24 hr against 3 changes of 4 l of distilled water at 4 °C; the other half was left in a test tube at 4 °C. After 24 hr, 1·0 ml of each mixture was test for MAO activity. The same experiment was performed simultaneously with harmaline at the same concentration ($1·5 \times 10^{-3}$ M). These experiments were performed twice with less than 5% variation.

* MAO activity could not be determined because harmaline absorbed too strongly at 360 mμ at this concentration; however, harmaline has been reported to inhibit MAO completely at concentrations much lower than this.[17]

TABLE 6. EFFECT OF PAD ON "SOLUBILIZED" MAO, AS COMPARED WITH THE MITOCHONDRIAL MAO

| Concentration of PAD | % Inhibition of mitochondrial MAO | % Inhibition of solubilized MAO |
|---|---|---|
| $2·5 \times 10^{-4}$ | 100 | 100 |
| $5·0 \times 10^{-5}$ | 74 | 74 |
| $1·0 \times 10^{-5}$ | 40 | 40 |

Cutscum was added to the mitochondrial suspension to make a 5% solution. It was shaken intermittently in the cold for 4 hr and then centrifuged at $100,000 \times g$ for 1 hr. The supernatant fraction was decanted and tested for MAO activity and inhibition by PAD. This was compared with the activity and inhibition obtained with another aliquot of the mitochondrial MAO. All assays were done in duplicate with less than 5% variation.

(5) *Effect of PAD on solubilized MAO.* It might be argued that, because of its detergent-like structure, PAD exerts a nonspecific solubilizing action on MAO. This was tested by observing the effect of PAD on MAO solubilized with Cutscum.[15] At three different concentrations of PAD, the percentage inhibition of the solubilized enzyme and of that in the mitochondrial suspension were the same (see Table 6), although the total activity of the solubilized enzyme was only one-fifth as great.

(6) *Ability of PAD to inhibit MAO* in vivo. PAD was administered intraperitoneally in various regimens to rats which were then sacrificed at varying times after the last dose. The range of dosages was from 10 to 60 mg/kg, for 1–7 days, and the elapsed time after the last administration of PAD was 1–5 hr. Brains and livers were treated as in (1). Little difference could be detected by this method in the activity of the homogenates on MAO of controls and treated animals, whereas animals given comparable dosages of the hydrazine inhibitors showed extensive or complete inhibition of MAO.

(7) *Effect of PAD on the levels of 5-HT in rat brain.* A sensitive indicator of MAO inhibition, *in vivo*, is an increase in the brain level of 5-HT. It was therefore of interest to determine whether PAD could raise this level. The results, shown in Table 7,

TABLE 7. EFFECT OF PAD ON 5-HT LEVELS IN BRAIN

| PAD (mg/kg) | No. of days administered | Change (%±) |
|---|---|---|
| 1. 30 | 1 | −15 ± 2 |
| 2. 15 | 7 | −36 ± 4 |
| 3. 20 | 2 | −40 ± 4 |
| 4. 50 | 2 | −64 ± 5 |
| 5. 65 | 1 | No change 24 hr after injection |

Drugs were given intraperitonally. Each group consisted of 6 male rats. In the first 4 experiments the treated animals and controls were killed by exsanguination 4 hr after PAD and their brains homogenized in 5 ml of 0·1 N HCl. In the fifth experiment the animals were killed 24 hr after administration of PAD.

indicate that PAD *lowered* rat brain levels of 5-HT when these were determined 4 hr after the last administration of PAD; 24 hr after a dose that produced extensive lowering of 5-HT in brain, the level had returned to normal.

(8) *Effect of PAD on the catecholamine levels of the rat brain.* No effect on brain catecholamines was observed when high doses of PAD were given intraperitoneally for either short or long periods.

(9) *Effect of PAD on 5-hydroxytryptophan decarboxylase of rat kidney* in vitro. PAD, $10^{-3}$ M, produced no significant inhibition of rat kidney 5-hydroxytryptophan decarboxylase.

(10) *Effect of PAD on 5-HT of mast cells.* The mast cells of the murine mastocytoma contain 5-HT in granules.[18, 19] In many obvious ways these cells are more suitable for studies on amine release than are the non-nucleated platelets that often are used. Table 8 shows that PAD and other long-chain quaternary pyridinium compounds, but not PAM, released 5-HT from mast cells. JB-516 also caused a similar release.

(11) *Effect of PAD and JB-516 on levels of 5-HT in rats after administration of reserpine.* It is conceivable that the release of 5-HT by PAD in the rat brain masked some inhibition of MAO that otherwise would have been detected through increased levels of 5-HT. It was thought that the administration of PAD, after maximal release of 5-HT by reserpine, might then permit the detection of MAO inhibition through the accumulation of newly synthesized 5-HT. Table 9 shows that inhibition of MAO could

not be demonstrated *in vivo* even by this method, whereas that caused by JB-516 is readily apparent.

(12) *Effect on rats of pretreatment with PAD followed by reserpine, and pretreatment with reserpine followed by PAD.* Pretreatment of rodents with an inhibitor of MAO can reverse the sedative effects of reserpine.[20] Since PAD possesses some of the properties of each of these compounds, it was of interest to observe the performance of

TABLE 8. RELEASE OF 5-HT FROM MAST CELLS

| Drug (0·5 mg/ml) | % Release |
|---|---|
| | 20 |
| PAD | 100 |
| Tetradecyl pyridine iodide | 100 |
| Cetyl pyridine iodide | 100 |
| PAM | 20 |
| JB-516 | 60 |

The mast cells were removed from DBA/2 mice by aspiration of the peritoneal contents, and suspended in Hank's medium.[26] Duplicates were incubated in 20-ml beakers at 37° for 2 hr in a Dubnoff shaker, with and without inhibitors dissolved in Hank's medium. Each beaker contained approximately $10^8$ cells. After incubation the cells were centrifuged and the supernatant fractions decanted. The cells were washed with more Hank's medium, centrifuged, and the 2 supernatant fractions combined. The 5-HT content of both the cells and the supernatant fractions were measured spectrophotofluorometrically. There was less than 5% variation in the duplicates.

TABLE 9. 5-HT LEVELS AFTER RESERPINE FOLLOWED BY EITHER PAD OR JB-516

| Compound administered | 5-HT levels (μg) | Range |
|---|---|---|
| Reserpine | 0·09 | 0·08–0·10 |
| Reserpine + PAD | 0·09 | 0·08–0·10 |
| Reserpine + JB-516 | 0·46 | 0·43–0·49 |

Eighteen rats were given 4 mg of reserpine per kg; 6 of these were given 15 mg of PAD per kg, and 6 were given 10 mg of JB-516 per kg, 5 hr after the reserpine. The 5-HT levels at 1 hr after the last injection were determined.

PAD in both roles. As can be seen in Table 10, the behavior of PAD was not typical of an inhibitor of MAO, since it did not reverse the sedative effects of reserpine when given as pretreatment; rather, it delayed them. On the other hand, PAD did not act like reserpine, since it did not produce hyperactivity in rats pretreated with JB-516.

(13) *Effects of various quaternary ammonium derivatives on MAO* in vitro. Of many readily available compounds tested, decamethonium (85% inhibition at $10^{-3}$ M), hexamethonium (25% at $10^{-3}$ M), and thiamine (30% at $10^{-2}$ M) exhibited weak inhibitory actions. Choline, acetylcholine, PAM, diphosphopyridine nucleotide,

N-methyl nicotinamide, thiamine pyrophosphate, succinyl choline, *d*-tubocurarine, stigmonene bromide, and neostigmine were inactive. Several short-chain N-alkyl derivatives of heterocyclic nitrogen compounds were also good inhibitors *in vitro*. These will be discussed in a future publication.

TABLE 10. EFFECT ON RATS OF PRETREATMENT WITH PAD FOLLOWED BY RESERPINE, AND OF PRETREATMENT WITH JB-516 FOLLOWED BY PAD

| Drug administered | Behavioral observations |
|---|---|
| Reserpine | Sedation in 20 min |
| PAD followed by reserpine | Sedation in 60 min |
| JB-516 followed by reserpine | Enhanced alertness and activity |
| JB-516 followed by PAD | No change from untreated animals |
| JB-516 | No change from untreated animals |

Each group consisted of 6 animals; these were kept in individual cages. Observations were continued for 4 hr. All doses (mg/kg) were: reserpine, 5; PAD, 50; JB-516, 20. PAD was given for 2 days, the last dose 4 days prior to reserpine or 30 min after JB-516. JB-516 was given 30 min before PAD or reserpine.

## DISCUSSION

In addition to its effects on acetylcholinesterase and cholinergic receptors, PAD irreversibly inhibited MAO *in vitro*, without preincubation. This sets it apart from the "hydrazines", which required preincubation, and from the harmala alkaloids, which are reversible inhibitors. The potency of PAD as a MAO inhibitor is considerable, and it is not unreasonable to predict that more potent inhibitors of MAO, containing a quaternary nitrogen, can be synthesized.

Every attempt to demonstrate inhibition *in vivo* was unsuccessful: there was no decrease in the MAO activity of liver and brain homogenates of PAD-treated animals; 5-HT levels in brain decreased rather than increased after treatment with PAD and were unchanged in rats pretreated with reserpine which might be expected to show an increase owing to MAO inhibition that could be obscured by simultaneous release; there was no reversal of the reserpine-induced depression in rats pretreated with PAD.

There are several questions to be considered in seeking an explanation for the absence of *in vivo*-inhibition of MAO. Can PAD penetrate the rat blood-brain barrier and reach the cells of its central nervous system? Can PAD enter the various types of cells of the rat liver? If penetration does occur, was the amount of PAD administered adequate to produce detectable inhibition?

The evidence for the ability of PAD to penetrate the rat blood-brain barrier is mainly the decrease in brain levels of 5-HT which it produces. (The ability of PAD to release 5-HT from rat brain without affecting catecholamines is a selectivity that few compounds possess). The release of 5-HT from mast cells *in vitro* by PAD supports the hypothesis that the release is mediated directly by this agent rather than in some indirect manner. There is the possibility that the releasing effect is produced by less PAD than is required for the inhibitory effect on MAO, so that sufficient PAD enters the brain to produce the former but not the latter effect. The exclusion of PAD from the rat liver is another possibility that cannot yet be dismissed, although it is not a very attractive one. The question of adequate dosage is less troublesome, as can

be shown by comparing PAD with iproniazid. After rats were given a single dose of iproniazid of $5 \times 10^{-5}$ moles/kg body weight, MAO activity determined subsequently *in vitro* did not return to normal for 5 days.[21] In the most vigorous attempt to demonstrate MAO inhibition by PAD, $1{\cdot}4 \times 10^{-4}$ moles of PAD was given to each of six rats daily for 7 days, and no inhibition on MAO of brain or liver was detected in any despite the evidence presented herein that PAD, like iproniazid, is an irreversible inhibitor, that PAD on a mole-for-mole basis is eight times more potent *in vitro* as an MAO inhibitor, and that the *in vitro*-assay used in these experiments[11] is more sensitive than is the one formerly in use.[21] Another possible explanation is that PAD is rapidly detoxified *in vivo* but not *in vitro* by the mitochondria. Thus, *in vivo*, PAD which reached amine-containing granules before reaching mitochondria could exert its releasing action, whereas the mitochondrial detoxification would prevent PAD from inhibiting MAO, a mitochondrial enzyme. If so, analogs of PAD that are not rapidly inactivated *in vivo* might be synthesized, and a useful MAO inhibitor might result. The possibility also exists of making the pyridine dodecyl derivative of a hydrazine resembling iproniazid, in this manner creating a molecule with two functional groups that inhibit MAO, and that is capable also of releasing 5-HT.

The weak ability of hexamethonium to inhibit MAO is of some interest in the light of the recent demonstration that inhibitors of MAO can produce ganglionic blockade.[22] This is not to suggest that the effects of hexamethonium are due to MAO inhibition; rather, it is meant to point up the reciprocity between these two activities that might lead to an exploration of other similarities. It is interesting, however, that hexamethonium potentiated the effects of 5-HT in some experiments with the isolated guinea pig ileum.[23]

In the same light, the many actions of PAD on both acetylcholinesterase and acetylcholine receptors, and on MAO may be reiterated. It would be of interest to determine whether the influences of PAD on the release of 5-HT and on the activity of MAO are of any importance for the understanding of the observations of Dettbarn and Wilson already alluded to.[5, 6] It is of note that chlorpromazine[24] and tolazoline[25] also inhibit both MAO and cholinesterase.

*Acknowledgements*—I wish to thank Professors N. J. Giarman, D. X. Freedman, A. D. Welch, and J. P. Green for supporting this work and providing valuable advice during its course. The work was supported by grants MY-3363 of the National Institute of Mental Health (U.S. Public Health Service) and the James Hudson Brown Memorial Fund of Yale University School of Medicine.

## REFERENCES

1. I. B. WILSON and S. GINSBURG, *Biochim. biophys. Acta* **27**, 196 (1958).
1. I. B. WILSON and S. GINSBURG, *Biochim. biophys. Acta* **18**, 168 (1955).
3. S. E. MAYER, R. MARCKEL and B. B. BRODIE, *J. Pharmacol. exp. Ther.* **127**, 205 (1959).
4. P. ROSENBERG, *Biochem. Pharmacol.* **3**, 212 (1960).
5. W. D. DETTBARN, *Biochim. biophys. Acta* **41**, 337 (1960).
6. W. D. DETTBARN, I. B. WILSON and D. NACHMANSOHN, *Science* **128**, 1275 (1958).
7. A. SJOERDSMA, T. E. SMITH, T. D. STEVENSON and S. UDENFRIEND, *Proc. Soc. exp. Biol., N.Y.* **89**, 36 (1955).
8. G. A. KNIGHT and B. D. SHAW, *J. chem. Soc.* 682 (1938).
9. S. UDENFRIEND, H. WEISSBACH and B. B. BRODIE, *Methods of Biochemical Analysis* Vol. VI, p. 95. Interscience, New York (1958).
10. A. BERTLER, A. CARLSSON and E. ROSENGREN, *Acta physiol. scand.* **44**, 273 (1958).
11. H. WEISSBACH, T. E. SMITH, J. W. DALY, B. WITKOP and S. UDENFRIEND, *J. biol. Chem.* **235**, 1160 (1960).

12. G. C. COTZIAS and J. J. GREENOUGH, *Nature, Lond.* **183**, 1732 (1959).
13. J. H. GADDUM and N. J. GIARMAN, *Brit. J. Pharmacol.* **11**, 88 (1956).
14. G. H. HOGEBOOM, *Methods in Enzymology* Vol. I, p. 16. Academic Press, New York (1955).
15. E. A. ZELLER, J. BARSKY, E. R. BERMAN, M. CHERKAS and J. R. FOUTS, *J. Pharmacol. exp. Ther.* **124**, 282 (1958).
16. J. P. GREEN and M. DAY, *Biochem. Pharmacol.* **3**, 190 (1960).
17. S. UDENFRIEND, B. WITKOP, B. G. REDFIELD and H. WEISSBACH, *Biochem. Pharmacol.* **1**, 160 (1958).
18. H. MELTZER. Unpublished observations.
19. P. HAGEN, R. J. BARRNETT and F. LEE, *J. Pharmacol. exp. Ther.* **126**, 91 (1959).
20. M. CHESSIN, E. R. KRAMER and C. C. SCOTT, *J. Pharmacol. exp. Ther.* **119**, 453 (1957).
21. E. ZELLER, *J. biol. Chem.* **214**, 267 (1955).
22. S. B. GERTNER, *J. Pharmacol. exp. Ther.* **131**, 223 (1961).
23. M. ROCHA E SILVA, J. R. VALLE and Z. P. PICARELLI, *Brit. J. Pharmacol.* **8**, 378 (1953).
24. F. T. EVANS, *Psychopharmacologia* **7**, 231 (1960).
25. J. J. LEWIS, *Introduction to Pharmacology* p. 170. Livingstone, London (1960).
26. W. F. SCHERER, *Amer. J. Path.* **29**, 113 (1953).