The treated animals died at an accelerated rate when compared to the controls.

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## Some Relationships Between Peripheral Monoamine Oxidase Inhibition and Brain 5-Hydroxytryptamine Levels in Rats.* (24969)

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Recently Sjoerdsma *et al.*(1) suggested *in vivo* assay in man of monoamine oxidase (MAO) inhibitors based on ability to suppress excretion of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-hydroxytryptamine (5-HT). Since rats excrete 5-HIAA(2), it seemed probable that the Sjoerdsma procedure could be modified into routine method of evaluation of *in vivo* peripheral MAO inhibition in the rat. In addition, by measuring the effect of same MAO inhibitors on brain 5-HT levels a comparison of peripheral with central effects would be obtained.

*Methods and materials. 5-HIAA excretion*: Male albino rats (175-225 g) were obtained from Carworth Farms. Compounds were administered orally to minimum of 6 rats. At various times after administration of inhibitor, 10 mg/kg of 5-HT (free base) was administered orally. Rats were then placed in metabolism cages and urine collected over subsequent 6 hours. Urines were stored in refrigerator overnight and analyzed next day. The nitroso-naphthal method(3) was employed to measure 5-HIAA levels with following modification: One ml of urine was placed in 50 ml glass-stoppered bottle containing 5 ml of water, to which was added 6 ml of 2,4-dinitrophenyl-hydrazine reagent. Optical measurements were performed with Beckman DU spectrophotometer and Pyrocell microcuvettes. *Brain 5-HT concentrations*: The procedure of Bogdanski *et al.*(4) was employed. 5-HT was determined with Farrand spectrofluorometer. The following test compounds were employed: Phenylisopropylhydrazine: PIH or JB-516 (Dr. J. Biel, Lakeside Labs); 2-isopropyl-1-isonicotinyl hydrazine: IPH or iproniazid (Dr. L. O. Randall, Hoffmann-LaRoche); harmine, harmaline, and tetrahydroharmine (Dr. F. H. Clarke, Schering Corp.); the authors are indebted to these investigators for these compounds.

*Results. 5-HIAA excretion*: Excretion of 5-HIAA by normal rats is 8.9 γ/100 g BW (95% confidence limits: 7-10.8 based on 18 determinations). Rats given 10 mg/kg of 5-HT orally will excrete 65.8 γ/100 g BW (95% confidence limits: 58.3-73.3 based on 83 determinations) or 5.6% of dose in urine as measurable 5-HIAA over 6 hours. Erspamer(2) reported that the rat excretes 5.5% of orally administered 5-HT (6 mg/kg) as 5-HIAA. Preliminary experiments indicated that inhibitor studies were more reliable when employing the exogenous 5-HT test rather than measuring only the endogenous 5-HIAA.

Fig. 1 indicates efficacy of various substances in diminishing excretion of 5-HIAA. The most potent compound was PIH. Harmine, harmaline, and tetrahydroharmine were somewhat less potent. Lysergic acid diethyl-

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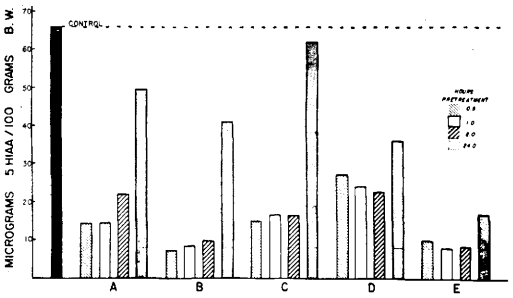


FIG. 1. Effect of harmine (A), harmaline (B), tetrahydroharmine (C), iproniazid (D), and JB-516 (E) on 6 hr excretion of 5-hydroxyindoleacetic acid in urine. Inhibitors (10 mg/kg) administered orally at times indicated prior to administering 10 mg/kg 5-hydroxytryptamine. Control excretion was 65.8 γ/100 g BW (95% confidence limits: 58.3-73.3).

amide and D-amphetamine (10 mg/kg PO), both of which are weak MAO inhibitors, *in vitro,* reduced 5-HIAA excretion from 65.8 γ to 53.6 and 57.1 γ/100 g BW respectively. Pentobarbital (10 and 35 mg/kg PO) was inactive as inhibitor of 5-HIAA excretion.

When doses of IPH less than 5 mg/kg were administered 0.5 hour prior to 5-HT, IPH was ineffective in decreasing excretion of 5-HIAA. A single dose of 5 or 10 mg/kg, however, reduced excretion of 5-HIAA from control level of 65.8 γ to 49.1 and 28.6 γ/100 g BW, respectively.

*5-HT brain levels.* Harmine, unlike PIH or IPH, did not markedly raise the level of 5-HT in brain when administered orally (Table I). However, when these drugs were administered by intraperitoneal route, harmine was as active as PIH and more active than IPH (Table I).

*Discussion.* The 5-HIAA excretion test appears to be sensitive and may be employed to evaluate MAO inhibitors. The most potent compound was PIH. Twenty-four hours after administration of PIH, administration of 5-HT was not followed by usual rise of 5-HIAA excretion in urine. No other agent tested was as effective as PIH. The potency of PIH agrees with that reported by Horita (5) and Biel *et al.*(6) employing other technics.

Comparison of harmaline, a potent MAO inhibitor(7), with PIH in the 5-HIAA excretion test indicates that these agents were approximately equiactive until the 24th hour. Differences at this time could be explained on the basis of rapid metabolism of harmaline(8) and relatively slow inactivation of PIH. Harmine, harmaline, and tetrahydroharmine appeared to be approximately equiactive.

Increase in 5-HT concentrations in brain after oral administration of PIH and harmine did not parallel the effects noted in the 5-HIAA excretion test. Although PIH was quite potent, harmine was relatively inactive. However, when given by intraperitoneal route, harmine considerably increased rat brain 5-HT concentration. This effect was equivalent to that noted previously with harmaline. It appears, therefore, that ineffectiveness of oral harmine in increasing the levels of brain 5-HT is due to lack of absorption from gastrointestinal tract or a more rapid inactivation when administered by this route.

Since 5-HT usually does not penetrate the "blood-brain barrier"(9), any blockade of 5-HT metabolism as measured in the 5-HIAA test is a manifestation of a predominant peripheral rather than central action. This type of specificity may be useful in broad evaluation of compounds. For example, pentobarbital, which increases brain 5-HT concentrations(10) does not inhibit excretion of 5-HIAA. The 5-HIAA method, therefore, of-

TABLE I. Increase (%) of Rat Brain 5-Hydroxytryptamine* after Administration of Monoamine Oxidase Inhibitors (10 mg/kg).

| Hr post Rx | 1 | | 2 | 24 |
|---|---|---|---|---|
| Route | Oral | Intraper. | Oral | Oral |
| Inhibitor | | | | |
| JB-516 (PIH) | 80 (6)†[71-90]‡ | 84 (6) [67-100] | 110 (6) [100-119] | 97 (12) [82-135] |
| Iproniazid (IPH) | 7 (6) [7] | 24 (12) [11-39] | 23 (6) [19-26] | 46 (6) [44-48] |
| Harmine | 9 (6) [8-10] | 79 (6) [76-81] | 27 (3) [27] | 0 (6) [0] |

* 5-HT concentrations for normals, 41-77.8 γ/g of brain, mean 54.6.
† Total No. of animals used.
‡ Range of values.

fers a means of detecting MAO inhibition directly, and when used in conjunction with other technics, serves to enlarge the pharmacology of various drugs.

*Summary.* A method for measuring activity of monoamine oxidase inhibitors as a function of suppression of 5-hydroxy-indoleacetic acid (5-HIAA) excretion is described. Increased excretion of 5-HIAA after 5-hydroxytyramine (5-HT) is effectively blocked by orally administered iproniazid, JB-516, and several harmala alkaloids. Correlation of these results with increased brain 5-HT is presented. Instances of non-correlation such as lack of effect of oral harmine on brain 5-HT with concomitant suppression of 5-HIAA excretion are discussed.

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## Induction of Plasma-Cell Neoplasms and Fibrosarcomas in BALB/c Mice Carrying Diffusion Chambers. (24970)

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Plasma-cell neoplasms are rare in mice (1,2). Rask-Nielsen(2) increased the incidence of these tumors in mice of 2 strains by injecting a carcinogen into the thymus. The findings reported here suggest that a new method of inducing plasma-cell tumors has been found. Seven plasma-cell tumors arose in mice in 2 experiments which were set up by Dr. Algire for another purpose. The tumors arose in BALB/c mice, a strain in which plasma-cell tumors have not been found previously. These induced tumors are of particular interest because at least 4 are transplantable and synthesize abnormal proteins. Six spindle-cell sarcomas also arose but similar tumors have been found in other experiments in association with diffusion chambers. Experiments are now in progress to obtain more information about induction of plasma-cell tumors, but since it will require a year or more to complete them, preliminary findings are reported now.

*Procedure.* Diffusion chambers were of the cell-impenetrable type described by Algire(3). The chambers were made of lucite rings and Millipore membranes which had pores averaging 0.10, 0.05 or 0.01 $\mu$ diameter. Tissue for placement in the chambers was obtained from spontaneous mammary tumors that arose in C3H mice carrying the milk agent. Two tumors were used, one in each experiment. A piece of tumor tissue about 0.5 mm diameter moistened with balanced saline containing tris buffer, horse serum, penicillin and streptomycin(3), was sealed into each chamber. The chamber was inserted into peritoneal cavity of agent-free BALB/c female mouse 5 or 6 weeks

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† Deceased.