Title

The effect of lysergic acid derivatives upon the ^{131}I distribution in 5-hydroxytryptamine challenged rats

Author

Daniels, Anne Elizabeth

Publication Date

1965

Peer reviewed|Thesis/dissertation

THE EFFECT OF LYSERGIC ACID DERIVATIVES UPON THE ^{131}I DISTRIBUTION IN 5-HYDROXYTRYPTAMINE CHALLENGED RATS

by

ANNE ELIZABETH DANIELS

A.B. San Francisco College for Women (1961)

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Comparative Pharmacology and Toxicology

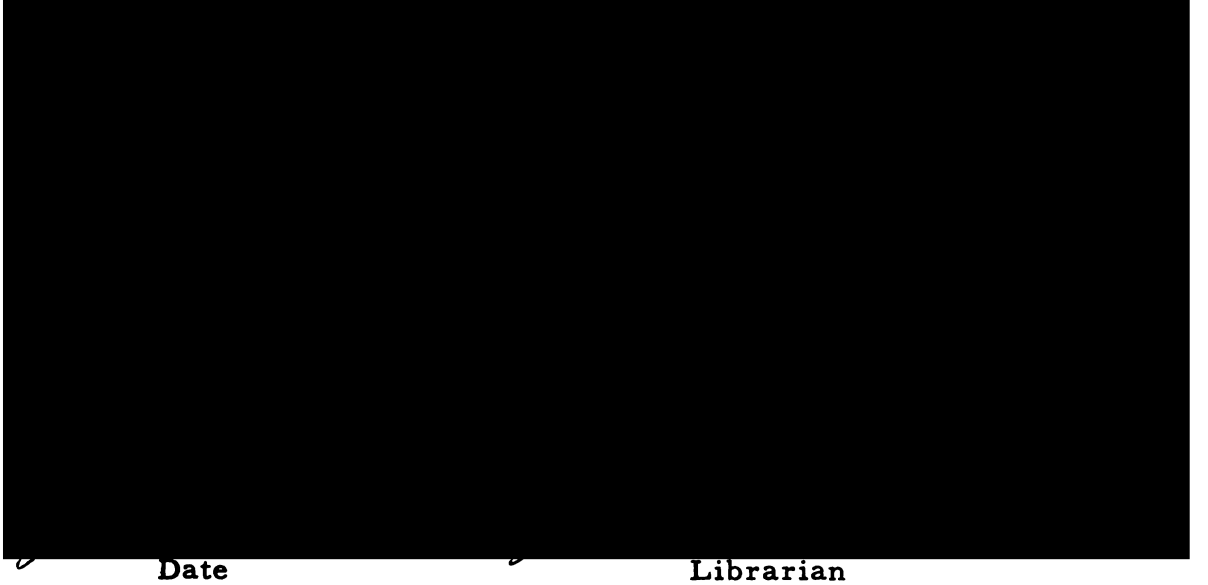
in the

GRADUATE DIVISION

[San Francisco]

of the

UNIVERSITY OF CALIFORNIA



Date Librarian

Degree Conferred: . . . . 10 June 1965 . . . . . .

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

The author wishes to express her gratitude to Professor Kenneth G. Scott for making this work possible. His assistance throughout the course of these studies is very much appreciated.

Financial aid supplied by a grant from the Henry, Laura and Irene B. Dernham Fund of the American Cancer Society, California Division, and by Cancer Research Funds of the University of California is gratefully acknowledged.

I am indebted to Mr. Harry Althouse of Sandoz Pharmaceuticals for generous supplies of MLD, UML, LPD, LSD, BOL, DAM, MBL, LAE, MPD and ALA; to the Lilly Research Laboratories for 21554, 23799, 23194, 24150, 23712 and 23877; to Dr. Z. Votava of the Research Institute of Pharmacy and Biochemistry in Prague, Czechoslovakia for CPA; and to Dr. I. Takeda of the Department of Biology, Kyushu University, Fukuoka, Japan for AGR.

To my mother and entire family I am also very grateful for their continual encouragement and assistance.

INTRODUCTION

Tumor-host relationships, or the systemic effects of tumors, constitute an important area of cancer research. These have been defined by Begg as "those changes produced in the tissues of the host remote from the tumor in which no evidence of metastatic malignant cells is found (1)."

The presence of a transmissible tumor alters the ^{131}I distribution of rats. This was first demonstrated in 1949 in studies on the fate of ^{131}I-tagged thyroglobulin (31). Subsequent research substantiated this finding (33,36,37). The chief characteristic was a reduced ^{131}I excretion and hence an increased ^{131}I tissue uptake. For this reason the phenomenon was called iodide trapping. The extent of the trapping was related to the size of the tumor. Initially, an increased uptake of iodine occurred in tissues adjacent to the tumor, but when the tumor reached about 1/250th of the body weight, systemic iodide trapping occurred. The deviation from normal ^{131}I distribution increased as tumor growth proceeded. A similar association between ^{131}I distribution and tumor growth has been demonstrated in humans with cancer (34). These patients had greater than normal plasma ^{131}I levels when the tumor mass represented less than 1/700th of the body weight. The deviation was proportional to the total tumor mass borne by the host in all species studied.

Further studies demonstrated that when the polypeptide fraction of a rat sarcoma was administered to normal animals, the iodide trapping effect was reproduced (32) suggesting that liberation of the polypeptide substance from the growing tumor might be responsible for the altered ^{131}I distribution described. Paton (26),

Norton and de Beer (21) and Bushby and Green (3) have reported that the administration of certain polypeptides resulted in the disruption of mast cells and the release of their contents. The tumor and/or its polypeptide were also shown to cause such mast cell disruption (35). These cells, which are widely distributed in the tissues of rats and mice, contain stores of histamine and serotonin (5-hydroxytryptamine, 5-HT) (2,25,40) which are released into the circulation when the cells are fragmented. In the human, only histamine has been associated with the granular material of these cells (24), but this has been recently challenged by Enerback who has shown that, at least under conditions of enhanced 5-HT secretion, human mast cells are capable of storing histochemically demonstrable amounts of 5-HT (9).

The administration of histamine and/or 5-HT to normal rats was shown to produce the same iodide trapping effect seen in tumor-bearing animals or in normal animals given tumor polypeptide. The iodide trapping was more prominent and occurred at lower dosages with 5-HT than with histamine (30). The same iodide trapping was obtained with other agents which cause amine release by disruption and degranulation of mast cells (30) and when these disrupting agents were given to rats <u>after</u> tumor implantation, or when the two amines were administered after tumor implantation, the growth of transplantable rat tumors was accelerated (35). On the other hand, mast cell depletion <u>prior to</u> tumor implantation, or the administration of agents known to block 5-HT and/or histamine, depressed or prevented tumor growth. LSD (d-lysergic acid diethylamide), MLD (d-1-methyl-lysergic acid diethylamide) and BOL (d-2-brom-lysergic

acid diethylamide) were shown to have such anti-tumor activity in rats and mice (38) and to reverse the iodide trapping effect to a varying extent when given to 5-HT treated rats.

Therefore, since (a) iodide trapping occurs in rats bearing transmissible tumors, (b) 5-HT accelerates tumor growth, (c) 5-HT blockers such as LSD, MLD and BOL depress tumor growth, (d) iodide trapping occurs in rats treated with 5-HT, and (e) LSD, MLD and BOL reverse the iodide trapping effect in rats treated with 5-HT, the effect of compounds on ^{131}I distribution in 5-HT treated rats was suggested as a measure of the ability of the compounds to block the effects of 5-HT and to depress or prevent tumor growth. In this study, nineteen compounds, eighteen of which are lysergic acid derivatives, have been screened for their effects on ^{131}I excretion in rats treated with 5-HT.

MATERIALS AND METHODS

Each study was carried out on six to eight groups of three unfasted rats of the Slonaker or Sprague-Dawley strain. Within each experiment the animals were of the same strain and sex and approximately the same age and weight. The average weight of the rats was 175 grams.

The structures and chemical formulae of the compounds investigated are listed in Figure I. All compounds were refrigerated until immediately prior to use. Minimum quantities of 3 mg. were dissolved in Ringers solution which was prepared by the method of Umbreit et al (39). Ringers solution was used for all subsequent dilutions as well to obtain solutions of 20, 50 and 200 µg/cc. To 23194, 23799, 23712, 27585, MBL and BOL, which were not readily soluble in Ringers solution, several drops of 1N HCl were added and the solution subsequently was neutralized with $NaHCO_3$. AGR and CPA were dissolved in 0.4% tartaric acid. 5-HT, as the creatinine sulfate complex, was administered in 1.5 mg. doses of the free base.

Table I indicates the dosages employed in each experiment. Controls received injections of Ringers solution.

The rats were anesthetized with ether. 5-HT and the experimental compounds, in one cc. volumes, were injected 15 minutes apart, subcutaneously, on opposite sides of the rat in the lateral femoral area. Immediately following the second injection, each rat received 25 µc. of sodium-radioiodide (^{131}I) in a carrier-free solution by intraperitoneal injection. This quantity did not appreciably expand the iodide pool of the animal since it corresponds to only 2.5×10^{-4} µg iodine or 0.04% of the daily iodine uptake

of the thyroid of the rat.

The rats were placed in metabolism cages (three rats per cage) and supplied with water. After four hours, they were again anesthetized and 5 cc. blood samples were taken by cardiac puncture before the animals were killed with ether. Specimens were then removed and weighed wet.

Urine and feces were washed from the bottom of the cages and from the crocks with iodine-carrier solution which consisted of 0.1N sodium thiosulfate and 0.1N sodium hydroxide. The total volume, including the washings, was approximately 500 cc. After mixing thoroughly, two 5 cc. aliquots were removed.

All samples were assayed for ^{131}I with a gamma ray spectrometer using a 4" x 5" thallium-activated sodium iodide crystal. Recoveries were 92.2 $\pm$ 0.4% of the dose. The values were corrected proportionally to 100% recovery for comparison between groups.

To determine whether there were any differences in the distribution other than those in excretion, tissue ^{131}I uptakes were further corrected using the following formula: $\frac{a}{e} = c$, where
a = actual percent of the ^{131}I dose per gram or per organ,
e = 100 minus the percent of the ^{131}I dose excreted by the group,
and c = corrected percent ^{131}I dose per gram or per organ.

The data are expressed as Mean $\pm$ Standard Error (S.E.$=\sqrt{\frac{dev^2}{n(n-1)}}$). Comparisons between groups were done using the methods of Fisher (12). The range of the "true mean" was estimated as the 95% confidence limits for the control group and for the group treated with 5-HT only. Results are expressed as the percent of the dose of ^{131}I found in the controls.

RESULTS

LSD and several of its derivatives studied here are known to be 5-HT antagonists and all of the compounds have been studied in animals treated with 5-HT. The data to be presented here concerns their effects on ^{131}I excretion and their possible antagonism to 5-HT in relation to the effects shown by LSD.

I EFFECT OF 5-HT ON ^{131}I DISTRIBUTION

The effects of 1.5 mg. 5-HT on the four hour ^{131}I distribution of the rat are shown in Table II. This dose of 5-HT was found to cause maximal depression of ^{131}I excretion at four hours. Significant differences from control ^{131}I uptake ($p = .001$) are seen in all tissues except the thyroid ($p = 0.4$). The most outstanding difference is in excretion levels.

Urinary and fecal ^{131}I are reported together in these studies as total ^{131}I excretion since pilot studies showed fecal excretion to constitute less than 1% of the dose in controls, animals treated with 5-HT only and in those treated with 5-HT and 10 or 250 ug LSD. Fecal ^{131}I values for these groups were 0.64 ± 0.17, 0.54 ± 0.17, 0.40 ± 0.03 and 0.85 ± 0.22 percent of the dose in 7, 9, 6 and 3 experiments, respectively.

As shown in Table III, when the values from Table II were corrected for excretion as described in the methods, no differences were apparent in tissue uptake of ^{131}I by controls and 5-HT treated animals, except in the thyroid ($p = .001$). When the data are corrected in this manner, the amount of ^{131}I excreted is removed from the distribution, leaving 59.6% of the dose in the control

animal and 91.7% in the 5-HT treated one. Since a similar amount of the dose was taken up by the thyroids of both groups prior to the application of this correction, a difference between the thyroid values would be expected after the correction is made. However, with the other tissues, the values for control and 5-HT animals were significantly different prior to the correction. Therefore it would not be expected that the values would be so similar unless: (a) their differences prior to correction were chiefly a reflection of the amount of ^{131}I available for distribution after excretion and (b) their values were proportional to the amount excreted. This appears to be the case here. Plasma, gastrointestinal tract, skin and remains values in 5-HT treated animals are not significantly different from the controls following this proportional correction for excretion. Because of this, it appeared that the main effect of 5-HT on the ^{131}I distribution of the rat was on the excretion of the isotope.

II EFFECTS OF COMPOUNDS ON ^{131}I EXCRETION WHEN ADMINISTERED TO ANIMALS NOT TREATED WITH 5-HT

Figure II shows the percent of the ^{131}I dose which was excreted by animals treated with 200 µg. of each compound alone. The compound causing the greatest amount of the dose of ^{131}I to be excreted, 52.5%, was 27585. This was not significantly different from the average control value of 40.4%, nor were the values obtained for MPD, 23194, ALA, UML, 23712, LAE, BOL, CPA, 24150, LSD, 21554, MBL, 23877, 23799, DAM or MLD when statistical comparison was done using the methods previously described. Animals treated with LPD

and AGR excreted an average of 15.4 and 9.3% of the ^{131}I dose, respectively, values which were significantly different from the controls ($p = .01$ and $.02$, respectively).

III EFFECT OF COMPOUNDS ON ^{131}I EXCRETION WHEN ADMINISTERED TO RATS TREATED WITH 5-HT

In one set of experiments, rats were treated with 1.5 mg. 5-HT prior to the administration of the compounds and in another set, 5-HT was given after the compounds. Both types of experiment were conducted because pilot studies showed that animals excreted different amounts of ^{131}I when the time of administration of the compound in relation to that of 5-HT was reversed. Data from these experiments are presented in Figures III to VI. The dose of the compound is plotted on the abcissa versus the amount of ^{131}I excreted, expressed as the percent of the dose of ^{131}I excreted by the controls on the ordinate. Note that the ordinate of Figure III differs from that of Figures IV, V and VI. The dashed lines represent the response when 5-HT was administered prior to the compound and the solid lines represent the response when 5-HT was administered after the compound.

For simplicity of presentation, the compounds have been separated into four groups according to their effect on ^{131}I excretion compared to the effect of LSD when 5-HT was given after 50 µg. of the compound. These groups are: (a) LSD and those compounds showing ^{131}I excretion levels higher than that of LSD, (b) compounds having from 60 to 100% of the LSD effect, (c) compounds having from 20 to 60% of the LSD effect, and (d) compounds having less than 20% of the

LSD effect. Comparison was made at the 50 µg. dose level because this dose of LSD was shown to have the maximum effect in supressing the growth of tumors (6). Comparison was made with data obtained when 5-HT was administered after the compound because most 5-HT blocking agents have been reported to act competitively (22,23,42) and such antagonism is presumably accomplished by preventing fixation of the agonist to its site of action.

95% confidence limits of ^{131}I excretion for the controls are 93 to 107% and for those treated with 5-HT only are 16 to 24% of the ^{131}I dose. These levels are indicated by the shaded areas in Figures III to VI.

A. LSD AND THOSE COMPOUNDS SHOWING ^{131}I EXCRETION LEVELS HIGHER THAN THAT OF LSD

Figure III shows the effects of UML, MLD, BOL and LSD on ^{131}I excretion. At all doses tested, when 5-HT was administered <u>after</u> these compounds the animals excreted more ^{131}I than when it was administered prior to the compounds. In this set of experiments, animals given MLD (20, 50 or 200 µg), UML (50 µg.) and LSD (200 µg.) excreted more ^{131}I than the controls. Values obtained from both types of experiments were above 5-HT levels at all doses, except when 5-HT was given prior to 20 µg. of LSD or UML. These two values were within the 95% confidence limits for 5-HT.

Maximum ^{131}I excretion levels were seen at 200 µg. doses for all of these compounds whether 5-HT was given prior to or after them with the exception of UML and BOL which caused more to be excreted when 5-HT was given after 50 µg. than 200 µg.

B. COMPOUNDS HAVING FROM 60 TO 100% OF THE LSD EFFECT

Figure IV shows the effects of DAM, 21554, 24150 and MPD. No overall difference in response was observed in the two types of experiments with this group of compounds. However, at 200 µg. doses, all four compounds showed higher ^{131}I excretion levels when 5-HT was given prior to them than when it was given after. None of the values exceeded control levels, but many were at levels shown by 5-HT treated rats.

Maximum ^{131}I excretion levels occurred at 200 µg. for all of these compounds whether 5-HT was given prior to or after them with the exception of 24150 and MPD which caused more to be excreted when 5-HT was given prior to 20 µg. doses than 200 µg. doses.

C. COMPOUNDS HAVING FROM 20 TO 60% OF THE LSD EFFECT

Figure V shows the effects of ALA, MBL, 27585, LPD, LAE and 23877. No overall difference in response was observed in the two types of experiments with this group of compounds. However, at all doses of LPD animals excreted more ^{131}I when 5-HT was administered prior to it than when administered after it.

When 5-HT was given prior to 200 µg. LPD, ^{131}I excretion exceeded control levels by a small amount. At all doses, excretion levels in ALA treated rats were at levels shown by 5-HT treated rats. Those for 23799 were all below these levels.

Maximum ^{131}I excretion levels were seen at 200 µg. doses for all of these compounds whether 5-HT was given prior to or after them except with ALA, 27585, and 23877, where more was excreted by animals treated with lower doses in the following cases: 5-HT prior

to 20 µg. ALA, 5-HT after 50 µg. ALA, 5-HT after 20 µg. 27585, and 5-HT prior to 50 µg. 23877.

D. COMPOUNDS HAVING LESS THAN 20% OF THE LSD EFFECT

Figure VI shows the effects of CPA, AGR, 23712, 23194 and 23799. Rats treated with three of these five compounds, CPA, 23712 and 23799, excreted more ^{131}I at all three dose levels when 5-HT was administered prior to them than when it was administered after them. None of these compounds showed excretion levels which reached those of the controls. Excretion levels for 23712 were all below 5-HT levels with the exception of that observed with 20 µg. when 5-HT was given prior to it. This was at levels shown by 5-HT treated rats.

As can be seen from the sharp peaks in these curves, the compounds in this group caused maximal ^{131}I excretion when 20 µg. were given, except for 23194, 23799 and 23712 which caused more to be excreted when higher doses were given. This occurred in the following cases: 5-HT prior to 200 µg. 23194 and to 50 µg. 23799 and 5-HT after 200 µg. 23712.

DISCUSSION

Iodine is removed from the circulating blood mainly by the thyroid and kidneys (27). In the kidney it is filtered through the glomeruli and that which is not excreted is reabsorbed into the circulation by the tubules (41). The main effect of 5-HT on the ^{131}I distribution of the rat at four hours appears to be depression of the excretion of the isotope.

The action of 5-HT on renal function has been discussed in detail by Maupin (19). The work of Erspamer showed that 5-HT constricts the afferent glomerular vessels of the kidney, an effect that is visible even macroscopically, and this causes a decreased glomerular filtration rate and an increased reabsorption by the tubules (10,11). This could account for the decrease in the excretion of ^{131}I in rats treated with 5-HT. Such an antidiuretic effect has been confirmed more recently in studies measuring the quantity of urine excreted by rats treated with 5-HT (5,7). These investigators also studied several lysergic acid derivatives for their ability to block the effect of 5-HT by this method.

The structural similarity of 5-HT and LSD was the basis for the investigation of Gaddum (13) who described the 5-HT blocking ability of LSD in 1953. As shown in Figure I, both compounds have an indole nucleus and an attached ethylamine grouping which occurs as a free chain in 5-HT and as part of the ring system in LSD.

A very large number of methods have been used to test many different types of compounds for their 5-HT blocking ability, including direct methods on both <u>in vitro</u> and <u>in vivo</u> preparations as well as indirect methods. These have been summarized by Gyermek (17).

In the majority of these experiments, LSD was the only lysergic acid derivative tested. It is a potent 5-HT antagonist on many preparations and its effect on the rat uterus has been used extensively as a reference in the evaluation of other anti-5-HT compounds (17).

At least forty derivatives of lysergic acid have been synthesized. Many of these have been tested for anti-5-HT activity, but most of the published studies include only two or three of them. A summary of the results from all of these studies is beyond the scope of this discussion. However, many of the compounds studied here have been tested on the isolated rat uterus by Cerletti and Doepfner (4) and Rothlin (28) and in the rat paw edema test by Doepfner and Cerletti (8). The results from their studies are listed in Table IV, columns 1 and 2, with respect to the activity of LSD. Comparable data from other investigations has been included. The effects of these compounds on the ^{131}I excretion of 5-HT treated rats are expressed in the same manner in Column 3.

It is well known that agents with apparent activity <u>in vitro</u> are often inactive <u>in vivo</u> and vice versa. Among the many discussions of this point with regard to lysergic acid derivatives and anti-5-HT effects are those of Gaddum (14) and Gyermek (17). The differences in intensity of the antagonism of 5-HT effects shown by the compounds studied here, even when the same preparation was used exemplify this. Note the values obtained by different workers for BOL, DAM and 21554 on the isolated rat uterus preparation (column 1, Table IV). UML is the only compound showing approximately the same degree of activity in all three types of experiments.

Although the psychotogenic effects of these compounds are most probably not related to their anti-5-HT action, they are listed in column 4 of Table IV to illustrate the variation in the degree of intensity of an important effect of this type of compound which occurs with structural variations.

From previous studies on the anti-5-HT activity of lysergic acid derivatives, several conclusions were drawn regarding structure-action relationships. Gyermek has summarized these as follows: (a) lysergic acid monoalkylamides are generally slightly less potent than LSD, (b) lysergic acid cycloalkylamides are about as active as LSD on the rat uterus but are weaker in the paw edema test, (c) among the dialkylamides of lysergic acid, the diethylamide has the peak potency; other dialkylamides are three to four times less potent, (d) compounds in which the amino group is a member of a cycloalkyl ring are considerably less effective than LSD, (e) l-lysergic acid derivatives are much less potent than the corresponding d-compounds, (f) substitutions on the ring system of the lysergic acid derivatives, such as bromination at the 2-position or methylation at the 1-position, or both, resulted in compounds more potent than LSD, whereas other substitutions yielded less potent compounds, (g) saturation of a part of the ring of lysergic acid resulted in weaker compounds (17).

The structural similarities of the compounds studied here are summarized in Table V. It is apparent that the list does not include compounds fitting into every group of lysergic acid derivatives mentioned in Gyermek's summary about the relationships between their structure and 5-HT blocking ability. However, relationships between

the structure of the compounds listed and the effect of 50 µg. doses on ^{131}I excretion when 5-HT was administered after them (column 3, table IV) are in agreement with his conclusions with the following modifications: (a) Lysergic acid monoalkylamides were generally less potent than LSD unless they were substituted somewhere else on the ring system. Monoalkylamides in this study included ALA, LAE, CPA, 23877 and UML. Of these, UML, which is substituted elsewhere on the ring system (methyl group at the 1-position), appears to be less potent. (b) Lysergic acid cycloalkylamides showed variable potencies in relation to that of LSD in this study. 24150 and MPD were slightly less active, whereas LPD and CPA were much less active. (c) Among the dialkylamides of lysergic acid, the diethylamide was the most potent when there were no substitutions elsewhere on the ring system or when there was a single substitution. LSD, BOL and MPD, all diethylamides, were more potent then DAM, lysergic acid dimethylamide. MBL, a diethylamide with substitutions in two positions on the ring system, was less potent than DAM. (d) Compounds in which the amino group was a member of a cycloalkyl ring (MPD and LPD) were less potent than LSD. (e) Compounds which were either 2-brominated or 1-methylated, but not both, were more potent than LSD with the exception of MPD which was slightly less potent than LSD. MBL, which is both 1-methylated and 2-brominated showed less activity than LSD, as did ALA which is a 1-acetylated derivative. 1-methylated derivatives also appeared to be more effective than LSD in blocking 5-HT antidiuresis in the group of compounds studied by DeCaro (7) and Chodera (5).

Although the similarity between the structure-action relationships

derived from other experiments and from these data on ^{131}I excretion support the postulation that 5-HT blocking is responsible for the ^{131}I excretion levels above those of 5-HT, the specificity of the 5-HT blocking action of the lysergic acid derivatives is questioned for several reasons: (a) Some compounds caused more ^{131}I to be excreted when 5-HT was given prior to them than when 5-HT was given after them. This occurred with all doses of LPD, CPA, 23712 and 23799, as well as with at least one dose of all of the other compounds studied with the exception of UML, MLD, BOL, LSD and 23877. (b) ^{131}I excretion levels in rats treated with 5-HT and a compound exceeded control levels in several instances (i.e. 5-HT after 50 µg. UML, 200 µg. MLD, 200 µg. LSD and 5-HT prior to 200 µg. LPD). (c) ^{131}I excretion levels in rats treated with 5-HT and a compound were below 5-HT levels in many instances (see figures V and VI). (d) 20 µg. or 50 µg. doses of the compound caused higher ^{131}I excretion levels than 200 µg. doses when given to animals treated with 5-HT in many instances. This is especially apparent in Figure VI.

The first two observations seem to indicate that some of the compounds have an action of their own to increase ^{131}I excretion. The third observation may indicate that some of the compounds have an action of their own to decrease ^{131}I excretion. In minute amounts, LSD itself has been reported to potentiate the action of 5-HT on the isolated rat uterus (6). However, LPD and AGR were the only compounds of the series which appeared to depress ^{131}I excretion when 200 µg. was given to animals not treated with 5-HT. Therefore, it appears that these compounds interact with 5-HT in some way other

than competitive inhibition. The fourth observation seems to support the hypothesis that these compounds have multiple actions since the degree of the effect on ^{131}I excretion varied somewhat independently of the dose. Similar dose-response relationships were noted with several of the compounds in studies on their effect on tumor growth (38). For these reasons, the lysergic acid derivatives studied here appear to interact with 5-HT to affect ^{131}I excretion in several different ways.

According to Page (22,23) and Woolley (42), the majority of 5-HT blocking agents act competitively. Such blocking is presumably accomplished by preventing fixation of an agent like 5-HT to a given receptor site and/or by displacing 5-HT from those sites, exposing it to enzymes which will inactivate it. The heterogenicity of 5-HT receptor sites has been discussed by Gyermek (17), Gaddum et al (15,16) and Meier et al (20). Ability to block 5-HT would depend on the affinity of the blocking agent for those receptors.

Due to the fact that these studies were done on intact animals, it is impossible to relate the results to specific receptor site interactions. It is possible that either or both of the two competitive blocking mechanisms stated above could be operating in the kidney, but it appears that other actions of these compounds are also important, the mechanisms of which are not understood.

Further studies which are proposed to help elucidate these complex interrelations and determine whether lysergic acid derivatives are specific blockers of the effects of 5-HT on ^{131}I excretion include: (a) ^{131}I excretion tests with these compounds alone at other dose levels; (b) studies on the effect of the anti-

diuretic hormone from posterior pituitary extract given alone and with lysergic acid derivatives on ^{131}I excretion to determine whether these derivatives are affecting tubular reabsorption when the glomerular vessels are not constricted by 5-HT; and (c) studies on renal blood flow and/or renal vascular resistance changes with 5-HT alone, posterior pituitary extract alone and lysergic acid derivatives alone as well as with 5-HT or posterior pituitary extract.

However, these studies were initiated primarily due to the observations that 5-HT enhanced the growth of tumors and several lysergic acid derivatives known to block 5-HT were capable of depressing or preventing such growth. Because the iodide trapping syndrome, shown by tumor-bearing rats and reproduced in normal rats treated with 5-HT, could be reversed to varying degrees with those few lysergic acid derivatives, the effects of this series of compounds were studied on the ^{131}I excretion of the rat. From these studies, it appears that the 5-HT blocking activity of the lysergic acid derivatives may not be the only property of these compounds that is responsible for their effects on ^{131}I excretion, indicating that this may also be the case with regard to their anti-tumor activity. Much work is required before these hypothetical properties could be defined. However, it may be warranted if any of the compounds prove to be effective in depressing tumor growth. To determine if they are effective in this respect, studies in which those compounds which showed ^{131}I excretion levels above 5-HT levels are administered to rats prior to and/or after the implantation of tumors are proposed.

SUMMARY

5-HT and eighteen lysergic acid derivatives have been studied for their effects on the four hour ^{131}I excretion of the rat. 5-HT depressed these levels significantly. Lysergic acid derivatives alone had little effect, with the exception of LPD and AGR, which also depressed ^{131}I excretion levels. When these compounds were administered to rats treated with 5-HT, ^{131}I excretion levels were generally above those observed when 5-HT was given alond, indicating that these compounds were blocking the effect of 5-HT. Relationships between the structures of these compounds and their ^{131}I excretion levels in 5-HT treated rats were similar to those observed for these agents in other studies of 5-HT blocking ability. However, actions other than 5-HT blocking appear to be taking place as indicated by the variations in response observed with alterations in compound (1) administration time with respect to 5-HT, (2) dose, and (3) structure. Further experiments are proposed to clarify these undefined actions.

When ranked according to their effects when 50 ug. doses were administered to 5-HT treated rats, UML, MLD and BOL ^{131}I excretion levels were up to five times those seen with LSD. DAM, 21554, 24150 and MPD levels were from 80 to 90% of the LSD effect. ALA, MBL, 27585, LPD, LAE and 23877 levels were from 30 to 60% of the LSD effect. CPA, AGR, 23712, 23194 and 23799 levels were below 20% of the LSD effect.

TABLE I

DOSES EMPLOYED IN EXPERIMENTS

Group	5-HT mg. free base/rat	Compound µg.
1	0	0
2	1.5	0
3	1.5	20
4	1.5	50
5	1.5	200
6	0	200

TABLE II

EFFECT OF 1.4 mg. 5-HYDROXYTRYPTAMINE ON THE FOUR HOUR ^{131}I DISTRIBUTION OF THE RAT

TISSUE	CONTROLS % ^{131}I Dose		1.5 mg. 5-HYDROXYTRYPTAMINE % ^{131}I Dose			% Controls
	n*	Mean ± S.E.	n*	Mean ± S.E.	p	Mean ± S.E.
Plasma, %/cc.	46	0.60 ± 0.02	45	0.98 ± 0.04	<.001	163 ± 6
G.I. Tract	68	17.1 ± 0.50	64	26.1 ± 0.60	<.001	159 ± 6
Thyroid	65	6.0 ± 0.30	61	6.2 ± 0.30	.4	108 ± 4
Skin, %/gm.	68	0.51 ± 0.02	64	0.75 ± 0.02	<.001	155 ± 6
Remains, %/gm.	68	0.18 ± 0.01	64	0.31 ± 0.01	<.001	175 ± 5
Excretion	68	40.4 ± 1.50	64	8.3 ± 0.80	<.001	20 ± 2

*number of experiments (3 rats per experiment)

TABLE III

EFFECT OF 1.5 mg. 5-HT ON THE FOUR HOUR ^{131}I DISTRIBUTION OF THE RAT WHEN DATA ARE CORRECTED FOR EXCRETION

	Controls % ^{131}I dose	1.5 mg. 5-HT % ^{131}I dose
Plasma, %/cc.	1.01 ± 0.01	1.06 ± 0.01
G.I. Tract	29.4 ± 0.6	29.3 ± 0.8
Thyroid	10.6 ± 0.4	6.7 ± 0.2*
Skin, %/gm.	0.83 ± 0.02	0.79 ± 0.02
Remains, %/gm.	0.31 ± 0.02	0.34 ± 0.02

*Significantly different from controls

TABLE IV

POTENCY OF SOME LYSERGIC ACID DERIVATIVES WITH RESPECT TO LSD

COMPOUND	PERCENT OF EFFECT OF LSD			
	1. Rat Uterus 5-HT Antagonism	2. Rat Paw Edema**** 5-HT Antagonism	3. ^{131}I Excretion in 5-HT Treated rats▲	4. Psychotogenic Effect in Man *****
UML	660*	440	510	0
MLD	368*, 350***	91	430	33
BOL	103*, 50***	29	180	2
LSD	100	100	100	100
DAM	23*, 20***	12	90	23
21554	43*, 2**		90	11
24150			80	
MPD	130*		80	5
ALA	39*		60	7
MBL	533**	26	60	1
27585			50	
LPD	5*		40	10
LAE	12*, 12***	22	40	12
23877			30	
CPA			20	
AGR			20	
23712			20	
23194			3	
23799			3	

* Cerletti and Doepfner (4)

** Sandoz Laboratories (29)

*** Rothlin (28)

**** Doepfner and Cerletti (8)

***** Isbell et al (18)

▲ Rats treated with 1.5 mg. 5-HT after receiving 50 µg. of the compound.

TABLE V

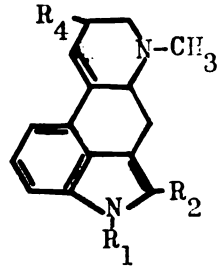
STRUCTURAL SIMILARITIES OF LYSERGIC ACID DERIVATIVES

R=4 SUBSTITUTIONS	
methyl	AGR
monoethylamide	LAE, ALA
diethylamide	LSD, BOL, MLD, MBL
cyclopentylamide	CPA, 24150
dimethylamide	DAM
pyrolidide	LPD, MPD
butanolamide	UML
morpholide	21554
hydroxy	23194, 23712, 23799
ethyl	23712
allyl	23799
sec-butylamide	23877
R=1 SUBSTITUTIONS	
acetyl	ALA
methyl	UML, MBL, MLD, MPD
R=2 SUBSTITUTIONS	
brom	MBL, BOL
R=4 AMIDE	UML, DAM, ALA, MPD, LSD, BOL, LAE, LPD, CPA, 24140 21554, 23877, MBL, MLD
RING STRUCTURE ON R=4 AMIDE	21554, 24150, CPA, LPD, MPD
Monoalkylamide	UML, ALA, LAE, CPA, 23877
Dialkylamide	LSD, BOL, MLD, MBL, DAM
non=amide	23799, 23194, AGR, 23712
Cycloalkylamides	
Amino group member of ring:	LPD, MPD
Amino group not in ring:	24150, CPA

FIGURE I

STRUCTURES OF COMPOUNDS STUDIED*

Lysergic Acid Derivatives



AGR $R_4 = CH_3$

ALA $R_4 = CONH\text{-}C_2H_5$; $R_1 = C{=}O$ with CH_3

BOL $R_2 = Br$; $R_4 = CON(C_2H_5)_2$

CPA $R_4 = CONH$-cyclopropyl

DAM $R_4 = CON(CH_3)_2$

LAE $R_4 = CONH\text{-}C_2H_5$

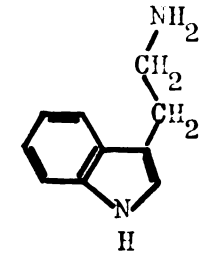
LSD $R_4 = CON(C_2H_5)_2$

MBL $R_1 = CH_3$; $R_2 = Br$; $R_4 = CON(C_2H_5)_2$

MLD $R_1 = CH_3$; $R_4 = CON(C_2H_5)_2$

MPD $R_1 = CH_3$; $R_4 =$ pyrrolidinyl-CO

5-Hydroxytryptamine (5-HT)



LPD $R_4 =$ pyrrolidinyl-CO

UML $R_1 = CH_3$; $R_4 = CONH\text{-}CH(C_2H_5)\text{-}CH_2OH$

21554 $R_4 = CONH$-morpholinyl

23194 $R_4 = CH(OH)CH_3$

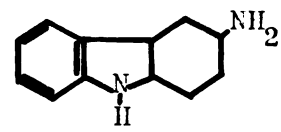
23712 $R_4 = CH(OH)C_2H_5$

23799 $R_4 = CH(OH)CH_2CH{=}CH_2$

23877 $R_4 = CONH\text{-}CH(CH_3)C_2H_5$

24150 $R_4 = CONH$-cyclopentyl-OH

27585



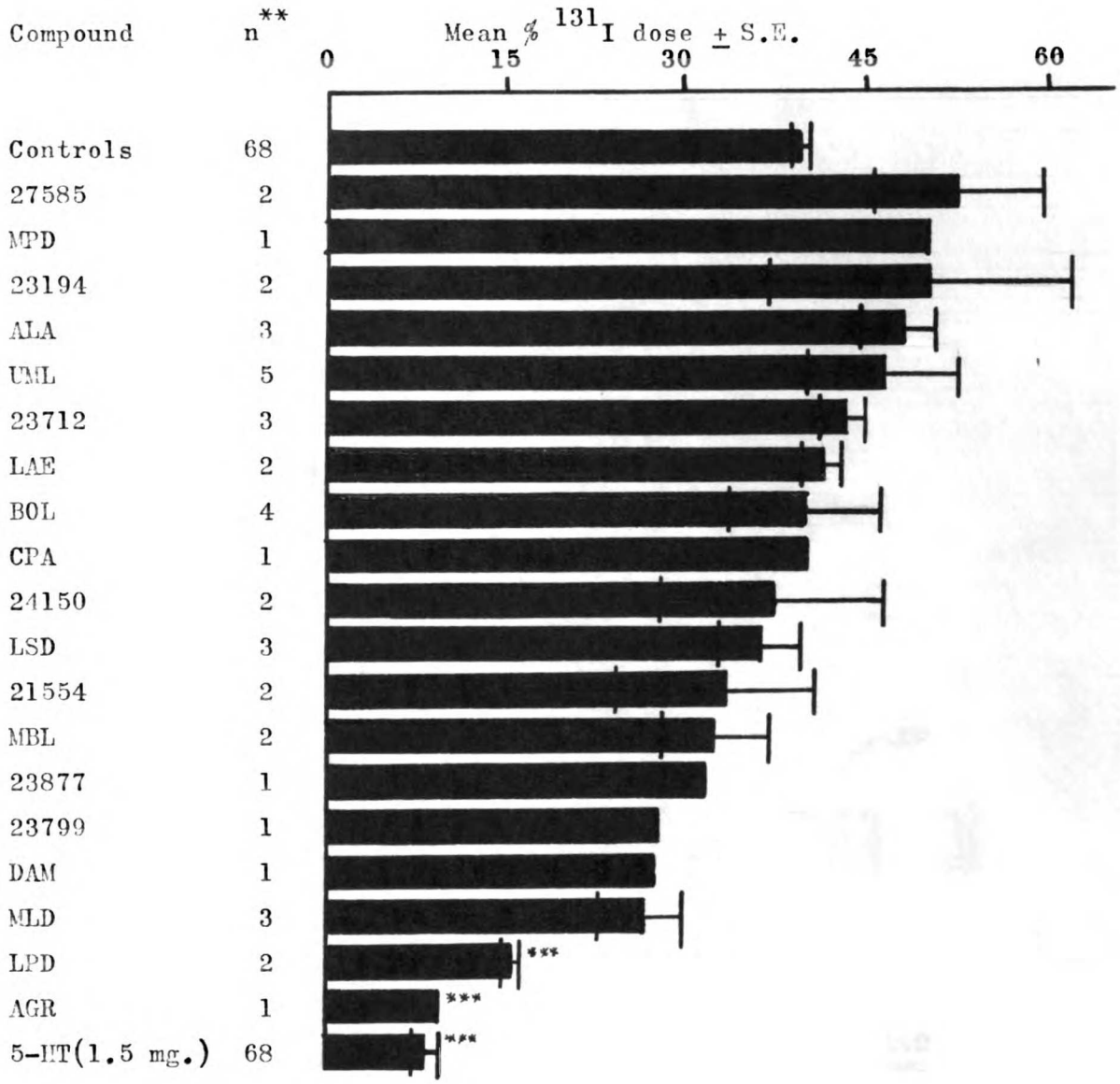
*Chemical formulae of compounds (see next page)

*Chemical formulae of compounds

AGR	Agroclavine; (7,9-dimethyl-4,5,5a,6,6a,7,8,9-octa-hydroindolo [4.3-fg] qunioline
ALA	d-l-acetyl-lysergic acid monoethylamide
BOL	d-2-brom-lysergic acid diethylamide
CPA	lysergic acid cyclopentylamide
DAM	d-lysergic acid dimethylamide
LAE	d-lysergic acid ethylamide
LSD	d-lysergic acid diethylamide
LPD	d-lysergic acid pyrrolidide
MBL	d-l-methyl-2-brom-lysergic acid diethylamide
MLD	d-l-methyl-lysergic acid diethylamide
MPD	d-l-methyl-lysergic acid pyrrolidide
UML	d-l-methyl-lysergic acid butanolamide
21554	d-lysergic acid morpholide
23194	7,9-dimethyl-9-hydroxy-4,5,5a,6,6a,7,8,9-octa-hydroindolo [4.3-fg] quinoline
23712	9-ethyl-9-hydroxy-7-methyl-4,5,5a,6,6a,7,8,9-octa-hydroindolo [4.3-fg] quinoline
23799	9-allyl-9-hydroxy-7-methyl-4,5,5a,6,6a,7,8,9-octa-hydroindolo [4.3-fg] quinoline
23877	N-(dl-sec-butyl) d-lysergic acid amide
24150	N-dl-trans-(2-hydroxycyclopentyl)-d-lysergic acid amide
27585	3-amino-1,2,3,4-tetrahydrocarbazole

FIGURE II

EFFECT OF COMPOUNDS ALONE ON ^{131}I EXCRETION*



* All compounds given in 200 µg doses.

** Number of experiments. Pooled excretion from 3 rats per experiment averaged.

*** Significantly different from controls.

FIGURE III

EFFECT OF LSD AND THOSE COMPOUNDS SHOWING ^{131}I EXCRETION LEVELS HIGHER THAN THAT OF LSD

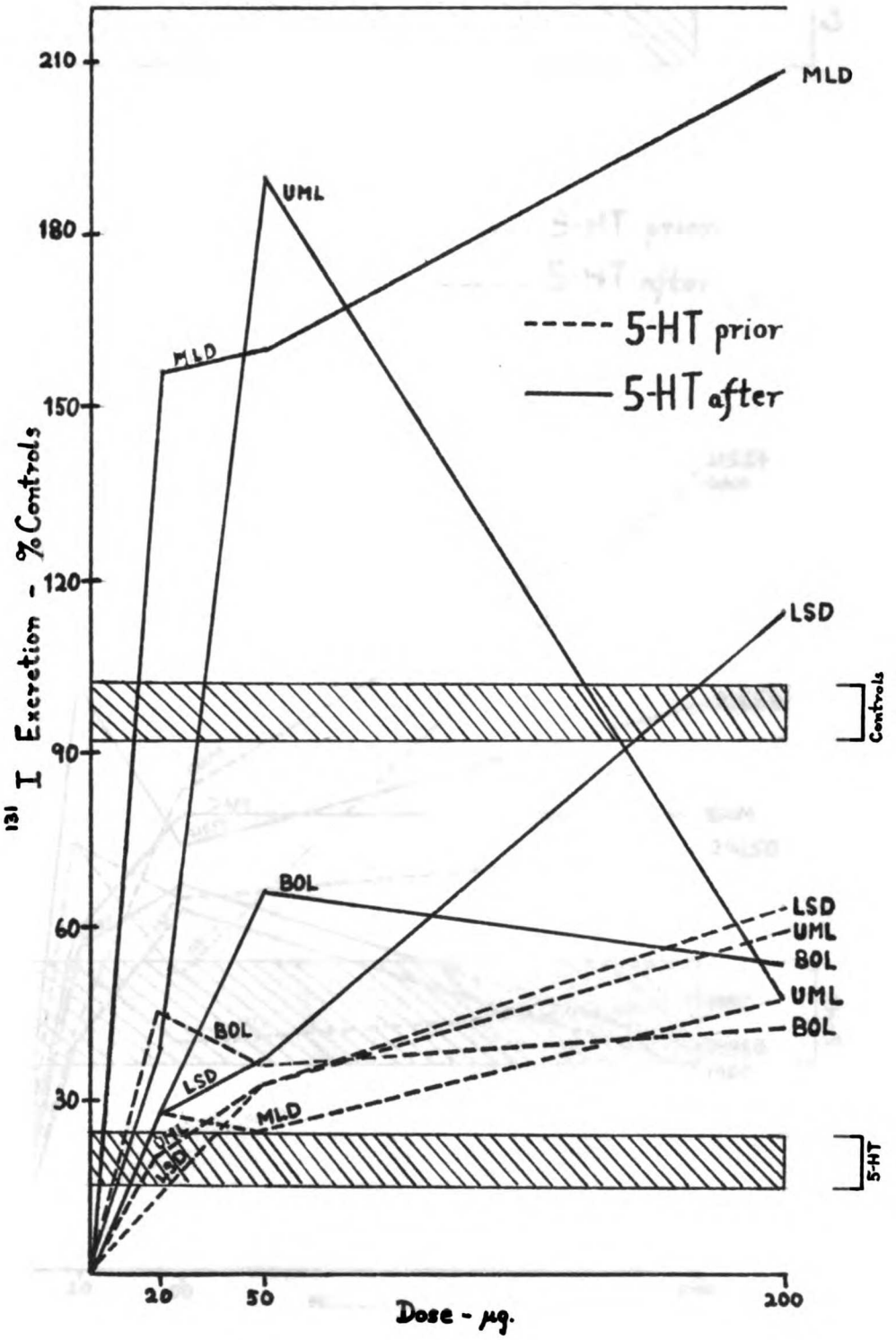


FIGURE IV

EFFECT OF COMPOUNDS HAVING FROM 60 TO 100%
OF THE LSD EFFECT

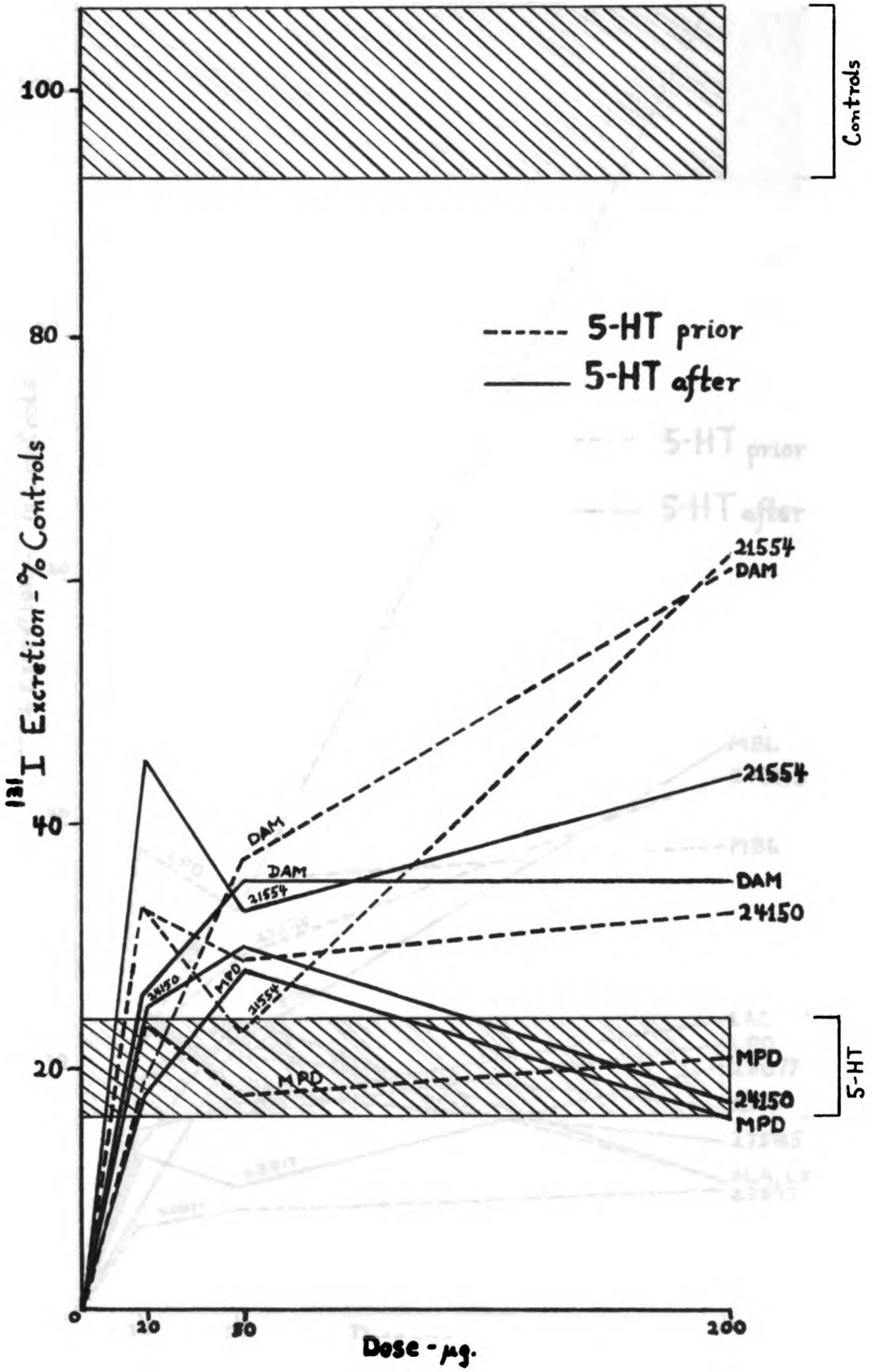


TABLE V
EFFECT OF COMPOUNDS HAVING FROM 20 TO 60%
OF THE LSD EFFECT

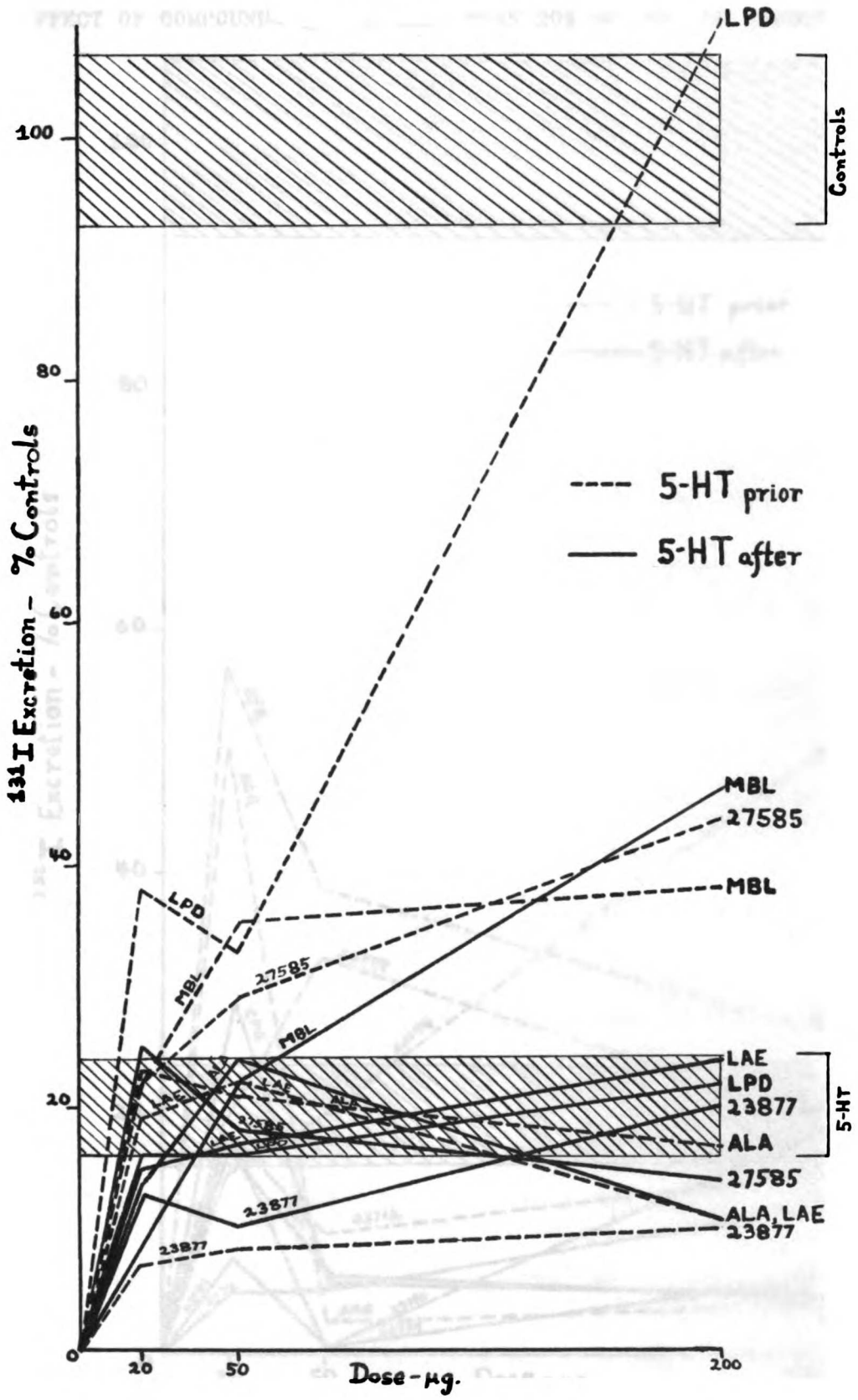
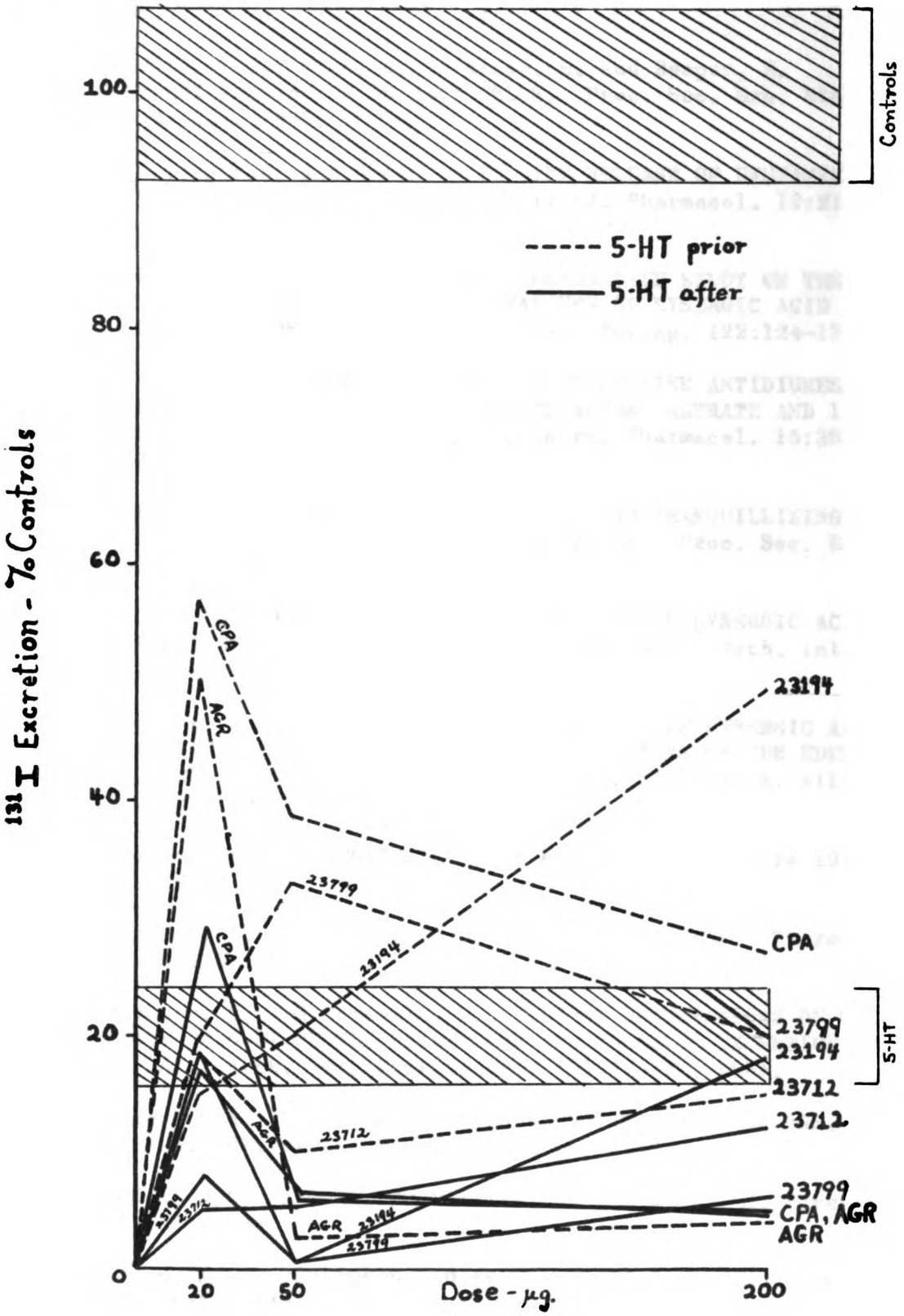


TABLE VI

EFFECT OF COMPOUNDS HAVING LESS THAN 20% OF THE LSD EFFECT



BIBLIOGRAPHY

1. Begg, R.W. TUMOR-HOST RELATIONS. Adv. Cancer Res. 5:1-54, 1954.

2. Benditt, E.P.; Wong, R.L.; Arase, M. and Roeper, E. 5-HYDROXYTRYPTAMINE IN MAST CELLS. Proc. Soc. Exp. Biol. Med. 90:303-304, 1955.

3. Bushby, S.R.M. and Green, A.F. THE RELEASE OF HISTAMINE BY POLYMYXIN B AND POLYMYXIN E. Brit. J. Pharmacol. 10:215-219, 1955.

4. Cerletti, A. and Doepfner, W. COMPARATIVE STUDY ON THE SEROTONIN ANTAGONISM OF AMIDE DERIVATIVES OF LYSERGIC ACID AND OF ERGOT ALKALOIDS. J. Pharmacol. Exp. Therap. 122:124-136, 1958.

5. Chodera, A. BLOCKADE OF 5-HYDROXYTRYPTAMINE ANTIDIURESIS IN RATS BY 2-BROMLYSERGIC ACID DIETHYLAMIDE TARTRATE AND 1-METHYL-LYSERGIC ACID BUTANOLAMIDE. J. Pharm. Pharmacol. 15:386-389, 1963.

6. Costa, E. EFFECTS OF HALLUCINOGENIC AND TRANQUILLIZING DRUGS ON SEROTONIN EVOKED UTERINE CONTRACTIONS. Proc. Soc. Exp. Biol. Med. 91:39-41, 1956.

7. De Caro, G. ANTAGONISTIC ACTION OF SEVERAL LYSERGIC ACID DERIVATIVES ON ANTIDIURESIS INDUCED BY 5-HT. Arch. int. Pharmacodyn. 141:54-61, 1963.

8. Doepfner, W. and Cerletti, A. COMPARISON OF LYSERGIC ACID DERIVATIVES AND ANTIHISTAMINES AS INHIBITORS OF THE EDEMA PROVOKED IN THE RAT'S PAW BY SEROTONIN. Int. Arch. Allergy 12:89-97, 1958.

9. Enerback, L. SEROTONIN IN HUMAN MAST CELLS. Nature 197:610-611, 1963.

10. Erspamer, V. PHARMACOLOGY OF INDOLEALKYLAMINES. Pharmacol. Rev. 6:425-439, 1954.

11. Erspamer, V. and Correale, P. FURTHER OBSERVATIONS ON THE ACTION OF 5-HT ON THE URINE FLOW AND CHLORIDE EXCRETION IN THE RAT. Arch. int. Pharmacodyn. 101:99-103, 1955.

12. Fisher, R.A. STATISTICAL METHODS FOR RESEARCH WORKERS. 10th ed., Oliver and Boyd, Edinburgh, 1948.

13. Gaddum, J.H. ANTAGONISM BETWEEN LSD AND 5-HT. J. Physiol. 121:15P, 1953.

14. Gaddum, J.H. SEROTONIN-LSD INTERACTIONS. Ann. N.Y. Acad. Sci. 66:643-648, 1957.

15. Gaddum, J.H. and Hameed, K.A. DRUGS WHICH ANTAGONIZE 5-HYDROXYTRYPTAMINE. Brit. J. Pharmacol. 9:240-248, 1954.

16. Gaddum, J.H. and Picarelli, Z.P. TWO KINDS OF TRYPTAMINE RECEPTOR. Brit. J. Pharmacol. 12:323-328, 1957.

17. Gyermek, L. 5-HYDROXYTRYPTAMINE ANTAGONISTS. Pharmacol. Rev. 13:399-441, 1961.

18. Isbell, H.; Miner, E.J. and Logan, C.R. RELATIONSHIPS OF PSYCHOTOMIMETIC TO ANTI-SEROTONIN POTENCIES OF CONGENERS OF LYSERGIC ACID DIETHYLAMIDE (LSD-25). Psychopharmacologia 1:20, 1959.

19. Maupin, B. SEROTONIN. DETERMINATION, METABOLISM, PHARMACOLOGY. SOME BIOLOGICAL ASPECTS. Psychopharmacol. Service Center Bull. December, p. 15-57, 1961.

20. Meier, R.; Tripod, J. and Wirz, E. CLASSIFICATION D'UNE SÉRIE D'ANTAGONISTES DE LA SÉROTONINE ET ANALYSE DE SES POINTS D'ATTAQUE VASCULAIRES PÉRIPHÉRIQUES. Arch. int. Pharmacodyn. 109:55-77, 1957.

21. Norton, S. and deBeer, E.J. EFFECT OF SOME ANTIBIOTICS ON RAT MAST CELLS IN VITRO. Arch. int. Pharmacodyn. 102:352-358, 1955.

22. Page, I.H. SEROTONIN. THE LAST FOUR YEARS. Physiol. Rev. 34: 563-588, 1954.

23. Page, I.H. SEROTONIN (5-HYDROXYTRYPTAMINE); THE LAST FOUR YEARS. Physiol. Rev. 38:277-335, 1958.

24. Parratt, J.R. p.136 in 5-HYDROXYTRYPTAMINE, ed. G.P. Lewis, Pergamon Press, London, 1958.

25. Parratt, J.R. and West, G.B. TISSUE HISTAMINE AND 5-HYDROXY-TRYPTAMINE. J. Physiol. 132:40P, 1956.

26. Paton, W.D.M. HISTAMINE RELEASE BY COMPOUNDS OF SIMPLE CHEMICAL STRUCTURE. Pharmacol. Rev. 9:269-328, 1957.

27. Riggs, D.S. QUANTITATIVE ASPECTS OF IODINE METABOLISM. Pharmacol. Rev. 4:284-354, 1952.

28. Rothlin, E. PHARMACOLOGY OF LYSERGIC ACID DIETHYLAMIDE AND SOME OF ITS RELATED COMPOUNDS. J. Pharm. Pharmacol. 9:569-585, 1957.

29. Sandoz Laboratories. Unpublished results.

30. Scheline, R.R. and Scott, K.G. MAST-CELL DISRUPTION AND I^{131} DISTRIBUTION IN THE RAT. Cancer Res. 18:932-937, 1958.

31. Scott, K.G.; Bostick, W.L.; Shimkin, M.B. and Hamilton, J.G. DISTRIBUTION OF GLOBULIN-BOUND THYROID FRACTIONS OF IODINE

IN NORMAL AND TUMOROUS ANIMALS AFTER INTRAVENOUS ADMINISTRATION USING RADIOIODINE AS A TRACER. Cancer 2:692-696, 1949.

32. Scott, K.G. and Daniels, M.B. RELATIONSHIP BETWEEN I^{131} METABOLISM, TUMOR GROWTH, AND REGRESSION. Cancer Res. 16: 784-787, 1956.

33. Scott, K.G. and Peng, C.T. TUMOR-HOST STUDIES: PHYSIOLOGICAL AND PHARMACOLOGICAL ACTION OF IODIDE-TRAPPING SUBSTANCE FORMED IN TUMOR-BEARING ANIMALS. Univ. Calif. Pub. Pharmacol. 2:345-376, 1955.

34. Scott, K.G.; Reilly, W.A. and Searle, G.L. I^{131} PLASMA AND THYROID LEVELS IN CANCER AND CONTROL PATEINTS. Cancer 13: 1261-1264, 1960.

35. Scott, K.G.; Scheline, R.R. and Stone, R.S. MAST CELLS AND SARCOMA GROWTH IN THE RAT. Cancer Res. 18:927-931, 1958.

36. Scott, K.G. and Stone, R.S. TUMOR-HOST STUDIES. II. INCREASED CONCENTRATION OF TAGGED IODOTYROSINES IN THE GASTROINTESTINAL TRACT OF RATS BEARING TUMORS. Cancer 3:722-724, 1950.

37. Scott, K.G. and Stone, R.S. TUMOR-HOST STUDIES. III. ALTERATION OF THYROID, SKIN, BLOOD AND TUMOR UPTAKE OF I^{131}-TAGGED DI-IODOTYROSINE IN RATS BY TRANSPLANTED TUMORS. Cancer 4:345-352, 1951.

38. Scott, K.G. and Stone, R.S. ANTI-TUMOR ACTION OF LYSERGIC ACID DERIVATIVES AND THEIR SEROTONIN-BLOCKING EFFECT AS REFLECTED BY I^{131} DISTRIBUTION IN RATS. Cancer Res. 19:783-787, 1959.

39. Umbreit, W.W.; Burris, R.H. and Stouffer, J.F.,p. 149 in MANOMETRIC TECHNIQUES, Burgess Pub. Co., Minneapolis, 1957.

40. West, G.B. 5-HYDROXYTRYPTAMINE, TISSUE MAST CELLS AND SKIN OEDEMA. Int. Arch. Allergy 10:257-275, 1957.

41. Williams, R.H., p. 101 in ENDOCRINOLOGY, 3rd ed., Saunders, Philadelphia, 1962.

42. Woolley, D.W. NEUROLOGIC AND PSYCHIATRIC CHANGES RELATED TO SEROTONIN. Prog. Neurobiol. 3:152-170, 1958.

COPY 1