THE 5-HYDROXYTRYPTAMINE CONTENT OF MOUSE BRAIN AND WHOLE MICE AFTER TREATMENT WITH SOME DRUGS AFFECTING THE CENTRAL NERVOUS SYSTEM

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(RECEIVED OCTOBER 15, 1959)

A number of drugs were examined for their ability to change the concentration of 5-hydroxytryptamine in mouse brain and in whole mice treated with 5-hydroxytryptophan. After β -phenylisopropylhydrazine or iproniazid, two inhibitors of monoamine oxidase, the brain 5-hydroxytryptamine rose to a maximum value in 8 hr., after which it declined, although a slight rise remained for as long as 6 days. Dose-effect relationships, determined 6 hr. after administration, showed β -phenylisopropylhydrazine to be approximately 60 times as effective as iproniazid in raising the brain 5-hydroxytryptamine. When mice were given 5-hydroxytryptophan and the amine content of the whole mice estimated, pretreatment with β -phenylisopropylhydrazine increased their 5-hydroxytryptamine content whereas pretreatment with iproniazid did not change it. The concentration of the amine in mouse brain and in whole mice was lower after reserpine, but was raised when reserpine and β -phenylisopropylhydrazine were given together. A small rise in brain 5-hydroxytryptamine was found after chlorpromazine; when chlorpromazine was given with iproniazid, however, the resulting increase was less than that found after iproniazid alone. Brain 5-hydroxytryptamine was unaltered after prolonged treatment with morphine.

Monoamine oxidase is widely distributed in animal tissues and is an important route for the inactivation of 5-hydroxytryptamine (Davison, 1958; Keglević, Supek, Kveder, Iskrić, Kečkeš, and Kisić, 1959). Effective inhibitors of monoamine oxidase should therefore retard the inactivation of the amine *in vivo*, but this is only partially true for 1-isonicotinyl-2-isopropylhydrazine (iproniazid), the most widely used inhibitor of monoamine oxidase. Although iproniazid is a strong and fairly specific inhibitor of this enzyme in homogenates (Zeller, Barsky, Fouts, Kircheimer, and Van Orden, 1952; Zeller, Barsky, and Berman, 1955), it has little effect on the inactivation of 5-hydroxytryptamine in tissue slices or in intact mice (Udenfriend, Weissbach, and Bogdanski, 1957). More recently, Horita (1958) has reported β -phenylisopropylhydrazine to be a potent inhibitor of monoamine oxidase. In the present experiments, both β -phenylisopropylhydrazine and iproniazid were examined as inhibitors of 5-hydroxytryptamine inactivation in intact mice.

5-Hydroxytryptamine is present in the nervous system of a wide variety of species (Amin, Crawford, and Gaddum, 1954; Correale, 1956), and this has led to much interest in possible relationships between 5-hydroxytryptamine and drugs which affect brain function (Gaddum, 1954; Woolley and Shaw, 1954; Brodie, Pletscher, and Shore, 1955; Paasonen and Vogt, 1956; Pletscher, 1957). Chlorpromazine, reserpine and morphine, three drugs with actions on the central nervous system, have therefore been included as test substances in some of the present experiments.

METHODS

Materials.—Male albino mice, of body weight 10 to 20 g., were maintained on cube diet 41B with free access to water. The drugs used were chlorpromazine hydrochloride, β -phenylisopropylhydrazine hydrochloride, iproniazid phosphate, reserpine, morphine sulphate, 5-hydroxytryptamine creatinine sulphate, ($\pm$)5-hydroxytryptophan, tryptamine hydrochloride, and ($\pm$)tryptophan. Quantities of tryptamine and 5-hydroxytryptamine have been expressed in terms of the base, but quantities of β -phenylisopropylhydrazine, iproniazid, morphine and chlorpromazine refer to the

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salts used. Drugs were freshly dissolved in distilled water, except reserpine, which was dissolved in a few drops of glacial acetic acid, diluted with 50 volumes of a mixture of equal parts of propylene glycol and ethyl alcohol, and made up to 100 volumes with distilled water. The stock solution of reserpine was diluted with distilled water immediately before use. Injections were given intraperitoneally in a dose volume of 0.1 ml./10 g. mouse, with the exception of 5-hydroxytryptophan, which was given in a dose volume of 0.2 ml./10 g. mouse.

Morphine Injected Mice.—Mice were injected daily with an increasing dose of morphine, under the supervision of Dr. S. J. Corne. The dose was increased as follows: 12.5 mg./kg. (10 days), 25 mg./kg. (21 days), 50 mg./kg. (7 days) and 100 mg./kg. (3 days). The final injection of morphine was given one hour before killing the mice and extracting 5-hydroxytryptamine.

Extraction of 5-Hydroxytryptamine.—Whole brains were dissected from mice which had been killed and bled out from the neck. The pooled brains from three mice were cut up finely with a scalpel on a glass slide and were extracted with 4 volumes of acetone (Analar). Next day, the acetone extract was separated from the residue and the residue was re-extracted overnight with 4 volumes of acetone. The second acetone extract was separated from the residue and the two extracts combined.

For the extraction of whole mice the acetone was acidified by the addition of 10 ml. *N* HCl/l. of acetone. Two mice were killed and homogenized with 4 g. sodium chloride and 4 volumes of acidified acetone. Next day the homogenate was filtered, and the residue extracted a second time overnight with 4 volumes of acidified acetone. On the following day the acetone extract was separated by filtration, and the residue on the filter paper washed with 30 ml. acidified acetone. The acetone extracts and washings were combined.

All extracts were kept at 0 to 4°. Just prior to the bioassay, an aliquot of acetone extract was evaporated to dryness at an external temperature of 35° and the residue re-extracted with a convenient volume of 0.9% sodium chloride.

Estimation of 5-Hydroxytryptamine.—The 5-hydroxytryptamine content of the saline extracts was determined on the isolated uterus of an oestrous rat. The uterus was suspended in a 15 ml. bath of oxygenated de Jalon solution at 29°. Atropine sulphate at a concentration of 10^{-6} was added to the de Jalon solution to increase the specificity of the preparation, which always responded to 0.02 to 0.03 μ g. 5-hydroxytryptamine.

Rat Fundus.—The rat fundus preparation was made as described by Vane (1957). The fundus strip was suspended in a 10 ml. bath of oxygenated Tyrode solution at 37°, and contractions were recorded from a pendular lever (Paton, 1957). Atropine sulphate was added to the Tyrode solution to give a concentration of 10^{-4} .

Paper Chromatography.—Acetone extracts were evaporated to dryness at 35°, and the residue extracted with 0.25 ml. *N* HCl and 3 ml. ether. After shaking, the ether layer was discarded, and the aqueous phase transferred to an evaporating dish with 2 ml. alcohol. The extract was concentrated to 0.1 to 0.2 ml. and applied to a sheet of Whatman No. 1 paper together with marker spots of tryptamine, 5-hydroxytryptamine and tryptophan. Chromatograms were developed by the descending technique with isopropanol/ammonia (880)/water (20:1:2), or with *n*-butanol/acetic acid/glacial/water (12:3:5). Occasionally two-way chromatograms were prepared with the same two solvent systems. All chromatograms were run for 5.5 to 7 hr. at a room temperature of 25.5 to 29.5°. Indoles were detected on the dried chromatograms by spraying with a 2% solution of *p*-dimethylamino-benzaldehyde in *N* HCl (Ehrlich's reagent), followed by heating at 80 to 85° for 3 to 5 min.

RESULTS

Identification of 5-Hydroxytryptamine in Extracts.—When bromolysergic acid diethylamide at a concentration of 10^{-7} was included in the de Jalon solution, the uterine responses to both 5-hydroxytryptamine and extracts were almost always abolished. Occasionally a brain extract still had a very slight stimulant action indicating the presence of a trace of interfering substance, but it was clear that the extracts reacted mainly with tryptamine receptors (Gaddum, 1953; Gaddum and Hameed, 1954).

Tryptamine has been identified in guinea-pig brain after administration of tryptophan and iproniazid (Hess, Redfield, and Udenfriend, 1959). Tryptamine and 5-hydroxytryptamine both react with tryptamine receptors and both are substrates of monoamine oxidase. The extracts were therefore examined for tryptamine, since this might have accounted for a significant part of their stimulant activity on the uterus. In extracts of mouse brains and whole mice 5-hydroxytryptamine was identified chromatographically as a blue spot with an R_F of 0.49 in butanol/acetic, and an R_F of 0.48 in isopropanol/ammonia. Tryptamine was not detected on these chromatograms. Even so, it seemed possible that tryptamine was present in small amounts. Tryptamine and 5-hydroxytryptamine can now be differentiated biologically, since Vane (1959) has shown that inhibitors of monoamine oxidase potentiate the response of the rat fundus preparation to tryptamine but not to 5-hydroxytryptamine. Tryptamine, 5-hydroxytryptamine, and brain extracts of normal mice and of mice treated with β -phenylisopropylhydrazine were tested on rat fundus preparations both before and after the addition of iproniazid 10^{-5} . After

FIG. 1.—Rat fundus. 10 ml. bath. Interval 5 min. T, tryptamine ($\mu\text{g.}$). HT, 5-hydroxytryptamine ($\mu\text{g.}$). X, brain extract from control mice (ml.). From IIH, iproniazid 10^{-5} was present in the Tyrode solution. Iproniazid potentiated the response to tryptamine, but did not potentiate the responses to 5-hydroxytryptamine or extract.

iproniazid, the response of a fundus to tryptamine was potentiated whereas the contractions produced by 5-hydroxytryptamine and the extracts remained relatively unaffected (Fig. 1). This was consistent with the view that the active material in the extracts was 5-hydroxytryptamine rather than tryptamine.

The Effect of Drugs on Uterine Response to 5-Hydroxytryptamine.—The following drugs were added to the organ bath at the same time as 5-hydroxytryptamine: 0.5 $\mu\text{g.}$ reserpine, 2 $\mu\text{g.}$ chlorpromazine, 30 $\mu\text{g.}$ iproniazid, 10 $\mu\text{g.}$ β -phenylisopropylhydrazine, and 100 $\mu\text{g.}$ morphine. The response to 5-hydroxytryptamine was reduced after chlorpromazine but was unaffected by the other drugs. When brain extracts of mice treated with chlorpromazine were tested there was no inhibition of the response to 5-hydroxytryptamine, however, showing that the extracts contained insufficient chlorpromazine to affect the uterus.

Recovery of 5-Hydroxytryptamine.—The extraction procedures adopted were similar to those of Correale (1956). The recovery of 5-hydroxytryptamine was tested by the addition of 0.5 $\mu\text{g.}$ 5-hydroxytryptamine to three brain extracts (1 g. of brain approximately) and 200 $\mu\text{g.}$ 5-hydroxytryptamine to two whole mice (30 g. approximately). In 5 determinations a mean of $73.2 \pm 2.0\%$ of the added 5-hydroxytryptamine was recovered from mouse brain, and, in 4

determinations, $64.5 \pm 5.2\%$ from whole mice. The recovery of added 5-hydroxytryptamine from whole mice remained unchanged at $63.0 \pm 7.6\%$ (4 determinations) when the mice were injected with β -phenylisopropylhydrazine (100 mg./kg.). Differences in the 5-hydroxytryptamine content of the extracts may be taken to represent differences in the 5-hydroxytryptamine content of the mice, since β -phenylisopropylhydrazine, an effective inhibitor of 5-hydroxytryptamine inactivation *in vivo*, did not alter the recovery of 5-hydroxytryptamine from the mice. Although Correale reported the recovery of 90–100% of added 5-hydroxytryptamine, other workers have obtained recoveries similar to those reported here, e.g. Twarog and Page (1953) recovered 60% of the added 5-hydroxytryptamine after extraction with acetone, and Welsh and Moorhead (1959) recovered 70% by a butanol extraction procedure.

The results given below have not been corrected for the loss of 5-hydroxytryptamine in the extraction procedure. A result has been taken as significant when P was less than 0.05.

TABLE I
INCREASES IN 5-HYDROXYTRYPTAMINE CONCENTRATION IN MOUSE BRAIN 1 TO 6 DAYS AFTER β -PHENYLISOPROPYLHYDRAZINE AND IPRONIAZID

In 7 control mice the brain 5-hydroxytryptamine concentration was 0.624 ± 0.099 $\mu\text{g./g.}$ brain.

Drug	Days after Administration of Drug	Mean Increase in 5-Hydroxytryptamine ($\mu\text{g./g.}$ Brain $\pm$ S.E.). No. of Determinations in Brackets	Significance of Mean from Zero (P)
β -Phenylisopropylhydrazine. (20 mg./kg.)	1	$+0.486 \pm 0.050$ (2)	<0.001
	2	$+0.063 \pm 0.050$ (2)	<0.25 , >0.1
	3	$+0.202 \pm 0.050$ (2)	<0.001
	4	$+0.169 \pm 0.045$ (2)	<0.001
	5	$+0.176 \pm 0.045$ (2)	<0.001
	6	$+0.128 \pm 0.045$ (2)	<0.01 , >0.005
Iproniazid (300 mg./kg.)	1	$+0.379 \pm 0.050$ (2)	<0.001
	2	$+0.146 \pm 0.050$ (2)	<0.005 , >0.0025
	3	$+0.311 \pm 0.050$ (2)	<0.001
	4	$+0.175 \pm 0.045$ (2)	<0.001
	5	$+0.187 \pm 0.045$ (2)	<0.001
	6	$+0.165 \pm 0.045$ (2)	<0.0025 , >0.001

5-Hydroxytryptamine in Mouse Brain.—Brain 5-hydroxytryptamine concentrations after administration of drugs have been expressed as differences between treated and control mice of the same experiment, the control values being given at the head of each table.

The effect of a single injection of β -phenylisopropylhydrazine (20 mg./kg.) or of iproniazid (300 mg./kg.) on mouse brain 5-hydroxytryptamine was examined over a period of days (Table I). One day after β -phenylisopropylhydrazine and iproniazid, increases in brain 5-hydroxytryptamine were 0.486 μ g./g. and 0.379 μ g./g. respectively, from a control value of 0.624 μ g./g. Even 6 days after these monoamine oxidase inhibitors, small but significant increases in brain 5-hydroxytryptamine were found.

Increases in mouse brain 5-hydroxytryptamine concentration after β -phenylisopropylhydrazine

(100 mg./kg.) and iproniazid (300 mg./kg.) were also investigated at more closely spaced time intervals over a period of 24 hr. (Fig. 2). The maximum increases in amine were found 8 hr. after administration of either compound. However, for practical reasons the effect of different doses of β -phenylisopropylhydrazine and iproniazid in raising the brain 5-hydroxytryptamine was investigated after 6 hr. (Fig. 3). The dose-effect curve for iproniazid had a flatter slope and a lower maximum than the corresponding curve for β -phenylisopropylhydrazine. It was not possible therefore to give an accurate ratio of the potencies for these two compounds. However, the effects of 5 mg./kg. β -phenylisopropylhydrazine and 300 mg./kg. iproniazid were of the same order, since they increased the brain 5-hydroxytryptamine by 0.347 μ g./g. and by 0.389 μ g./g., respectively.

FIG. 2.—Increases in brain 5-hydroxytryptamine in mice after treatment with β -phenylisopropylhydrazine (100 mg./kg.), marked $\bullet$ — $\bullet$, or iproniazid (300 mg./kg.), marked $\circ$ — $\circ$. The mean value for 5-hydroxytryptamine in control brains was 0.497 ± 0.032 μ g./g. (17 determinations). Ordinate, increase in brain 5-hydroxytryptamine (μ g./g.). Abscissa, interval between injection of compound and extraction of 5-hydroxytryptamine (hr.). Each point is a mean value $\pm$ S.E.

Table II shows differences in the 5-hydroxytryptamine concentration of mouse brain 6 hr. after the intraperitoneal injection of various drugs. The concentration of amine was lower after reserpine (2 mg./kg.), was higher after β -phenylisopropylhydrazine (20 mg./kg.), and was increased by the combination of these two drugs at the same dose levels. When all the data were combined as in Table II, the rise in 5-hydroxytryptamine after β -phenylisopropylhydrazine was higher than that after reserpine plus β -phenylisopropylhydrazine. However, when the comparison was restricted to results determined in parallel, the difference between the two groups was not significant ($P > 0.2$). A small but significant rise was found in each of 9 experiments after chlorpromazine (20 mg./kg.), but the increase in brain 5-hydroxytryptamine after chlorpromazine plus iproniazid was significantly less than the increase found after iproniazid alone ($P < 0.02$, > 0.01). No significant difference in 5-hydroxytryptamine concentration was found between the brains of control mice and mice treated with morphine.

5-Hydroxytryptamine in Whole Mice Injected with 5-Hydroxytryptophan.—Table III shows the effect of some drugs on the 5-hydroxytryptamine extracted from mice injected with 5-hydroxytryptophan. All mice received 5-hydroxytryptophan (150 mg./kg.), and 3 hr. later were killed and extracted for

FIG. 3.—Increases in brain 5-hydroxytryptamine in mice 6 hours after varying doses of β -phenylisopropylhydrazine, marked $\bullet$ --- $\bullet$, and iproniazid, marked $\circ$ - - - $\circ$. The mean value for 5-hydroxytryptamine in control brains was 0.449 ± 0.029 $\mu\text{g./g.}$ (10 determinations). Ordinate, increases in brain 5-hydroxytryptamine ($\mu\text{g./g.}$). Abscissa, doses of β -phenylisopropylhydrazine and iproniazid (mg./kg.), plotted on a logarithmic scale. Each point is a mean value $\pm$ S.E.

5-hydroxytryptamine. Drugs tested in the mice were administered intraperitoneally at specified periods before injecting the mice with 5-hydroxytryptophan. Treatment with reserpine (2 mg./kg.) lowered the 5-hydroxytryptamine concentration in mice; in animals treated with β -phenylisopropylhydrazine (100 ml./kg.), it was increased above control levels, but values after treatment with iproniazid (300 mg./kg.) were not significantly different from the controls. 5-Hydroxytryptamine was also increased when β -phenylisopropylhydrazine and reserpine were injected together; indeed, in mice injected with β -phenylisopropylhydrazine plus reserpine it was not significantly different from β -phenylisopropylhydrazine treated mice ($P > 0.2$).

TABLE II

CHANGES IN 5-HYDROXYTRYPTAMINE CONCENTRATION IN MOUSE BRAIN 6 HOURS AFTER INTRAPERITONEAL INJECTION OF VARIOUS DRUGS

In 18 control mice the brain 5-hydroxytryptamine concentration was 0.478 ± 0.027 $\mu\text{g./g.}$ brain.

Drug	Dose (mg./kg., i.p.)	Changes in 5-Hydroxytryptamine ($\mu\text{g./g.}$ brain $\pm$ S.E.), No. of Determinations in Brackets	Significance of Mean from Zero (P)
Reserpine	2	-0.264 ± 0.012 (6)	< 0.001
Reserpine + β -phenylisopropylhydrazine	2 20	$+0.512 \pm 0.012$ (6)	< 0.001
β Phenylisopropylhydrazine	20	$+0.746 \pm 0.076$ (12)	< 0.001
Iproniazid	300	$+0.445 \pm 0.057$ (11)	< 0.001
Iproniazid + Chlorpromazine	300 20	$+0.286 \pm 0.065$ (9)	< 0.005 , > 0.002
Chlorpromazine	20	$+0.108 \pm 0.021$ (9)	< 0.001
Morphine	(See methods)	-0.001 ± 0.025 (3)	> 0.9

DISCUSSION

Both β -phenylisopropylhydrazine and iproniazid caused an accumulation of 5-hydroxytryptamine in mouse brain. Between 2 and 8 hr. after β -phenylisopropylhydrazine (100 mg./kg.) or iproniazid (300 mg./kg.), the rise of 5-hydroxytryptamine in mouse brain was very rapid, 1.12 $\mu\text{g./g.}$ brain accumulating after β -phenylisopropylhydrazine. The accumulation of 5-hydroxytryptamine in brain after the monoamine oxidase inhibitors showed that it was synthesized at a high rate; it was calculated that the normal brain content accumulated in about 2.5 hr. after β -phenylisopropylhydrazine. The amine reached its maximum concentration in brain 8 hr. after β -phenylisopropylhydrazine or iproniazid and then declined. This decline may have been due to re-activation or re-formation of brain monoamine oxidase. Complete regeneration of brain

TABLE III
THE EFFECT OF SOME DRUGS ON
5-HYDROXYTRYPTAMINE LEVELS IN
WHOLE MICE TREATED WITH
5-HYDROXYTRYPTOPHAN

All mice received 5-hydroxytryptophan (150 mg./kg.) 3 hr. before extraction of 5-hydroxytryptamine. In 8 control mice the concentration of 5-hydroxytryptamine was 3.91 ± 0.26 $\mu\text{g./g.}$ mouse.

Drug (hr. Before Injection of 5-Hydroxytryptophan)	Dose (mg./kg., i.p.)	5-Hydroxytryptamine ($\mu\text{g./g.}$ Mouse $\pm$ S.E.). No. of Determinations in Brackets	Significance of Difference Between Means of Treated and Control Mice (<i>P</i>)
Reserpine (3 hr.)	2	2.76 ± 0.25 (3)	<0.05 , >0.02
Reserpine (3 hr.) + β -phenylisopropylhydrazine (1 hr.)	2 100	6.37 ± 0.75 (3)	<0.0025 , >0.001
β -Phenylisopropylhydrazine (1 hr.)	100	7.73 ± 0.64 (6)	<0.001
Iproniazid (1 hr.)	300	3.94 ± 0.68 (4)	>0.90

monoamine oxidase was a lengthy process, however, since the brain 5-hydroxytryptamine was still slightly raised after 6 days.

When different doses of β -phenylisopropylhydrazine and iproniazid were compared for their ability to raise the brain 5-hydroxytryptamine concentration, 6 hr. after administration of drug, β -phenylisopropylhydrazine was found to be about 60 times as effective as iproniazid, since 5 mg./kg. β -phenylisopropylhydrazine and 300 mg./kg. iproniazid both produced a rise of 0.3 to 0.4 $\mu\text{g.}$ 5-hydroxytryptamine/g. brain. A similar order of effectiveness was found for these two compounds when they were examined as *in vitro* inhibitors of monoamine oxidase (Udenfriend, Witkop, Redfield, and Weissbach, 1958). The present results therefore support the view that the rise in 5-hydroxytryptamine in brain after β -phenylisopropylhydrazine and iproniazid was due to inhibition of brain monoamine oxidase.

Log dose-effect curves were obtained by plotting increases in brain 5-hydroxytryptamine against doses of β -phenylisopropylhydrazine or iproniazid. Sigmoid curves were obtained for both com-

pounds, but the curve for iproniazid had a flatter slope and a lower maximum than the curve for β -phenylisopropylhydrazine. The lower maximum of its curve suggested that iproniazid might inhibit both formation and inactivation of 5-hydroxytryptamine in the brain. According to Davison (1956), a metabolite of iproniazid inhibits enzymes requiring pyridoxal phosphate. This supports the present interpretation of the log dose-effect curve for iproniazid, since pyridoxal phosphate is a co-enzyme of the decarboxylase which forms brain 5-hydroxytryptamine.

Inhibition of 5-hydroxytryptamine inactivation by β -phenylisopropylhydrazine must be widespread in the tissues of the mouse, since the 5-hydroxytryptamine content of animals injected with 5-hydroxytryptophan was increased after treatment with β -phenylisopropylhydrazine (100 mg./kg.). This was a severe test of the effect of β -phenylisopropylhydrazine, since the substrate was presented to the enzyme *in vivo*; iproniazid tested in the same way was without effect at 300 mg./kg.

After reserpine (2 mg./kg.), 5-hydroxytryptamine was reduced by 63% in mouse brain and by 30% in whole mice. When β -phenylisopropylhydrazine was given with reserpine, the 5-hydroxytryptamine concentrations of mouse brain and whole mice were raised, suggesting that monoamine oxidase is involved in the disappearance of 5-hydroxytryptamine after reserpine. The 5-hydroxytryptamine content of mice injected with 5-hydroxytryptophan and β -phenylisopropylhydrazine remained unaltered when the mice were pretreated with reserpine. This showed that reserpine (2 mg./kg.) had no appreciable effect on the decarboxylation of 5-hydroxytryptophan, which is in agreement with Brodie, Tomich, Kuntzman and Shore (1957). The difference between the increase in brain 5-hydroxytryptamine found after reserpine plus β -phenylisopropylhydrazine and that found after β -phenylisopropylhydrazine alone was not significant when the comparison was made between results determined in parallel.

The increase in brain 5-hydroxytryptamine concentration found after iproniazid plus chlorpromazine was significantly less than the increase found after iproniazid alone. This suggested a depression of 5-hydroxytryptamine formation after chlorpromazine. According to West (1958), chlorpromazine inhibits the 5-hydroxytryptophan decarboxylase of rat kidney *in vivo*. Such a decarboxylase inhibitor would be expected to cause a fall in brain 5-hydroxytryptamine within

a few hours, since the rate of formation of 5-hydroxytryptamine in brain has been shown to be high. After chlorpromazine, however, 5-hydroxytryptamine concentration in the brain was slightly raised above the corresponding control values. The reason for the small rise in brain 5-hydroxytryptamine after chlorpromazine is not understood. Possibly chlorpromazine inhibited some route of 5-hydroxytryptamine inactivation. According to Weissbach (1958), 5-hydroxytryptamine can be inactivated by cytochrome oxidase, an enzyme which is known to be inhibited by chlorpromazine *in vitro* (Bernsohn, Namajuska and Boshes, 1956; Dawkins, Judah, and Rees, 1959).

Costa and Himwich (1958) found a lowered 5-hydroxytryptophan decarboxylase activity in rabbit brain after convulsive doses of insulin, although the concentration of brain 5-hydroxytryptamine was raised or unaltered. Their results suggest that insulin, like chlorpromazine, depresses turnover of cerebral 5-hydroxytryptamine.

I would like to thank Professor G. A. H. Buttle for permission to work at the London School of Pharmacy, and Dr. G. B. West for his encouragement and welcome advice. I am also indebted to Dr. H. O. J. Collier for helpful suggestions, to Mrs. J. Skerry for technical assistance, to Dr. T. F. Grey for the β -phenylisopropylhydrazine, and to Dr. B. T. Warner for statistical analysis.

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