DHE 496/LSD 81/Hyg
Quart.J.Exper.Physiol., London 40, 49 (1955).

QUANTITATIVE STUDIES OF ANTAGONISTS FOR 5-HYDROXYTRYPTAMINE.

By J. H. GADDUM, KHAN A. HAMEED, D. E. HATHWAY and F. F. STEPHENS. From the Department of Pharmacology, University of Edinburgh, and Glaxo Laboratories Ltd., Greenford.

(*Received for publication* 12*th November* 1954)

IN a previous paper [Gaddum and Hameed, 1954] we have summarized earlier work on antagonists for 5-hydroxytryptamine (HT) and described experiments with known drugs. The present paper gives the results of a study of the actions of a number of indole compounds in order to discover active antagonists. It was necessary to measure their potencies, and our attention was thus drawn to some of the difficulties which arise when antagonists are compared quantitatively with one another.

Rough measurements may be made by finding the dose necessary to abolish the effect of the active drug (the "agonist") completely. Such measurements are unlikely to be very accurate since the result will depend on the dose used, and on the sensitivity of the record to small effects produced in the presence of the antagonist. Our own experience of this type of experiment confirms the impression that it is not very reliable. It is probably more satisfactory to use a method depending in some way on a measurement of the effect produced by the antagonist, and it is with such methods that this paper is mainly concerned.

When the effect of a given dose of agonist is inhibited, it is generally found that larger doses are still effective, but can be blocked by larger doses of the antagonist. Such results are sometimes described by calling the antagonism competitive, but simple experiments of this type do not prove the theory that the two drugs are competing with one another for the same receptor in the tissue. Some other word is needed to describe the observation that an increase of dose of either drug overcomes the effect of the other. It is proposed to call antagonisms of this type surmountable, and to include in this group the antagonisms which have been called competitive, non-competitive and uncompetitive [Chen and Russell, 1950], as well as those which depend on the two drugs combining with one another to form an inactive complex [Gaddum, 1943]. Examples of unsurmountable antagonisms will be discussed later.

The Measurement of the Effect of the Antagonist

The effect of the antagonist may be measured in terms of the percentage reduction of the recorded response to the agonist. This is only possible in a limited range of doses of the antagonist and is unlikely to give constant results. When experiments are made on isolated organs, measurements are therefore generally made of the following quantities:

A_0: the concentration of the agonist which has a given effect (y) in the absence of the antagonist.

A: the concentration of the agonist which has the same effect in the presence of the antagonist in concentration B and at time t.

When the antagonism is surmountable, the dose-ratio (A/A_0) is often taken as a measurement of the effect produced by the antagonist. Sometimes it is independent of y, so that the log–dose–effect curves before and after the antagonist are parallel. This is, however, not always so [Schild, 1949]. Cases are known in which A/A_0 increases with y, so that the antagonist makes the log–dose–effect curve flatter, and cases are also known where the reverse is true. The quantity A/A_0 can then only be used as an expression of the effect of the antagonist if y is kept constant, and Schild [1949] has proposed that it should be 50 per cent of the maximum effect.

Rocha e Silva and Beraldo [1948], Beraldo and Rocha e Silva [1949] and Rocha e Silva [1950] have suggested that the effect of an antagonist should be measured in terms of the time the muscle takes to recover when the antagonist is removed from the bath. They have studied this recovery in some detail and use more than one way of expressing their results, but the simplest one is R50, which is the time in seconds for the muscle to recover sufficiently to give a contraction half as great as the original contractions. When the experiments are confined to one drug, this time increases with the dose and can be used as a measure of the effect of the antagonist. There is no evidence that the use of this quantity avoids the complications discussed above due to the fact that A/A_0 is not independent of y. Rocha e Silva *et al.* avoid such complications by using a constant dose of the agonist.

The importance of this kind of measurement is emphasized by the work of Fleckenstein [1952], who found that the actions of specific antagonists often lasted longer than those of less specific drugs. On the other hand, duration of action is not the only property of drugs which may be worth measuring. Mepyramine antagonizes the action of histamine in the guinea-pig's ileum in lower doses than promethazine, but its action does not last so long [Reuse, 1948]. If the metameter proposed by Rocha e Silva *et al.* were used as the only criterion of the action of antagonists, such differences would be obscured. In any case,

some drugs have such a prolonged action that the use of this method of investigation would be impossible [Gaddum, 1926].

For these reasons the ratio A/A_0 is probably a more generally useful measure of the effect produced by an antagonist.

The Measurement of the Potency of Antagonists

The potencies of antagonists may be compared by measuring, for each, the concentration needed to have a standard effect (A/A_0) in a standard time. This is the basis for the calculation of the pA_x, which is the negative logarithm of the molar concentration, where A/A_0 is x [Schild, 1947]. This quantity only gives the potency in arbitrarily restricted conditions, but it has been found to give surprisingly constant results in different laboratories [Reuse, 1948]. The concentration of an agonist needed to have a standard effect may vary enormously, but the concentration of an antagonist for a standard effect seems to vary much less.

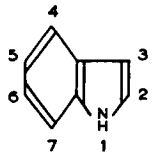
The pA_2 provides a measurement of the threshold concentration which just produces a small effect. The pA_{10} can generally be estimated more accurately, and the conditions are such as to ensure that the antagonist is fairly potent.

To estimate the pA_x it is necessary to expose pieces of tissue to various concentrations of the antagonist and to estimate in each case whether x is greater or less than the required value. Schild [1947] estimated the pA_2 by first getting a constant effect with a constant dose of agonist alone, and then testing the effect of double this dose in the presence of a suitable concentration of the antagonist, and observing whether the effect produced at the standard time was greater or less than the original effect. The concentration for equal effects was then estimated by interpolation. This is probably the most satisfactory method of making accurate comparisons between antagonists, but may be a laborious way of studying the effects of a long series of drugs whose properties are at first quite unknown, and whose effects may increase almost indefinitely with the time of exposure. Other methods of estimating the potency of antagonists are discussed later.

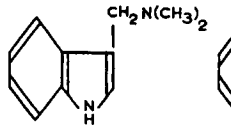
Methods

Rat's uterus in œstrus was found by Erspamer [1952] to be particularly sensitive to 5-hydroxytryptamine (HT), and this tissue has been used in practically all the experiments described here. Stilbœstrol (0·1 mg. per kg.) was injected the day before the experiment, but ovariectomy was not found necessary. The technique was the same as that described in the previous paper [Gaddum and Hameed, 1954]

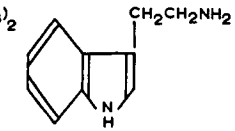
The volume of the bath was 2 ml. This preparation was chosen for this work mainly because simple indole compounds, such as gramine, were found to depress the response to HT in concentrations which did not depress the response to other drugs. When tested on other tissues, such as the guinea-pig's ileum, the effect of gramine appeared to be less specific, and when sufficient concentrations were used to depress the response to HT, the response to other drugs was also depressed. In the experiments described here acetylcholine was applied as a control drug in doses causing submaximal effects similar to those due to HT. The



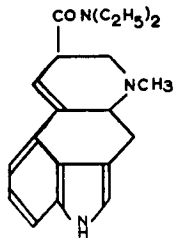
INDOLE



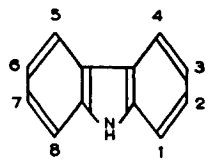
GRAMINE



TRYPTAMINE



LSD



CARBAZOLE

statement that the effect was specific has the arbitrary meaning that the response to HT was depressed by doses of the antagonist which did not affect the response to acetylcholine. No other control drugs were used. In order to avoid possible errors due to the persistence of effects a separate piece of uterus was used for each experiment.

The specific antagonists shown in Table II were all either immediately soluble, or were easily dissolved by the addition of a small amount of HCl. Most of the less active drugs shown in Table III could not be dissolved in this way, but were dissolved in a small volume of ethanol. When this solution was diluted with de Jalon's solution the drugs generally remained in solution, but more ethanol was added to redissolve them when necessary. The possible effects of this ethanol are discussed below.

The concentrations of the various drugs are given in terms of the weight of the base.

Most of the drugs used in these experiments were specially synthesized

for this purpose. The sources of some of the other drugs have already been acknowledged [Gaddum and Hameed, 1954]. We are especially grateful to Dr. K. K. Chen for hypaphorine and cinobufotenine, to Dr. Harley Mason for adrenolutin and to Dr. T. B. B. Crawford for adrenochrome. The other compounds in Table I were supplied by L. Light & Co. Ltd.

Many of the compounds were prepared by methods already given in the literature. The following compounds are new.

Methyltryptamines.—The 4-, 5-, 6- and 7-methyltryptamines were prepared from the corresponding methylindoles by the route: (*a*) Mannich reaction to methylgramines [Rydon, 1948; Supniewski and Scrafin-Gajewski, 1940], (*b*) quaternization with dimethylsulphate to give gramine metho-sulphates [Schöpf and Thesing, 1951], (*c*) reaction of the quaternary salts with aqueous potassium cyanide to give the 3-indolyl acetonitriles [Thesing and Schülde, 1952], and (*d*) reduction of the nitriles to tryptamines with lithium aluminium hydride.

6-Methylindol-3-ylacetonitrile.—6-Methylgramine (5 g.) dissolved in tetrahydrofuran (33 ml.) and glacial acetic acid (0·4 ml.) was methylated by addition to dimethylsulphate (16·7 g.) dissolved in tetrahydrofuran (14 ml.) and glacial acetic acid (0·4 ml.). The addition, which took 10 minutes, was accompanied by vigorous stirring and the temperature kept at 10°–15°. After the mixture had been kept at room temperature for 3 hours in the dark, the quaternary salt was obtained as a white crystalline solid (7·1 g.), of indefinite m.p., by filtration, washing with ether and drying. The metho-sulphate was heated for 1 hour at 65°–70° with potassium cyanide (4·4 g.) and water (70 ml.), during which the *nitrile* separated in a crystalline form and was finally removed by filtration, washed and dried to give 3·6 g., m.p. 112°–114°. Recrystallization from benzene-light petroleum (40°–60°) or aqueous ethanol gave plates, m.p. 114°–114½° (Found: C, 77·8; H, 5·88; N, 16·35. $C_{11}H_{10}N_2$ requires C, 77·8; H, 5·89; N, 16·43 per cent.).

In a similar manner the following methyl 3-indolyl acetonitriles were prepared: *2-methylindol-3-ylacetonitrile,* needles, m.p. 83° (benzene-light petroleum) (Found: N, 16·6 per cent); *4-methylindol-3-ylacetonitrile,* needles, m.p. 107°–108° (benzene-cyclohexane) (Found: N, 16·8 per cent); *5-methylindol-3-ylacetonitrile,* needles, m.p. 88°–89° (light petroleum, b.p. 100°–120°) (Found: C, 77·57; H, 5·96; N, 16·5 per cent); *7-methylindol-3-ylacetonitrile,* plates, m.p. 125°–126° (light petroleum, b.p. 100°–120°—ethyl acetate) (Found: C, 77·54; H, 5·86; N, 16·81 per cent).

4-Methyltryptamine.—4-Methylindol-3-ylacetonitrile (1 g.) dissolved in ether (40 ml.) was slowly added to lithium aluminium hydride (*ca.* 1 g.) in ether (25 ml.). The mixture was stirred for 1½ hours, the complex decomposed with water (5 ml.) then sodium hydroxide (30 ml., 2N) added. The ether layer was separated, the aqueous phase again

extracted with ether and the combined dried ether evaporated to give crude *4-methyltryptamine* (1 g.), m.p. 116°–117°. Recrystallization from benzene-*cyclo*hexane gave clusters of white needles, m.p. 118°–119° (Found: N, 16·03. $C_{11}H_{14}N_2$ requires N, 16·07 per cent). Picrate, m.p. 260° (*decomp.*) (bright red needles from ethanol).

7- and 5-Methyltryptamines.—A solution of 7-methylindol-3-ylacetonitrile (1·7 g.) in ether (150 ml.) was added dropwise to a solution of lithium aluminium hydride (*ca.* 2 g.) in ether (150 ml.) and the resulting mixture boiled under reflux for 2 hours. The complex was decomposed with ice and some of the aluminium hydroxide dissolved by addition of 2N sodium hydroxide; the remaining alumina was removed by filtration. After extraction with ethyl acetate (200 ml.) the aqueous phase was discarded and the combined solvents washed with water. The base was taken into hydrochloric acid (400 ml., 2N) by repeated extraction and the solvent layer discarded. Basification of the acid phase with sodium hydroxide (400 ml., 2N) liberated the free base, which was taken into ether (500 ml.) by repeated extraction. Evaporation of the dried ether (anhydrous magnesium sulphate) left solid *7-methyltryptamine* (1·02 g.). On washing the crude product (0·85 g.) with peroxide-free ether, 7-methyltryptamine (0·6 g.), m.p. 120°–122° was obtained. An alcoholic solution (10 ml.) of picric acid (0·5 g., 2·1 mols.) was refluxed for 10 minutes with crude 7-methyltryptamine (0·17 g.) and, after cooling, the orange-red *picrate* was removed and washed with ethanol. The *picrate* crystallized from ethanol in blood-orange prisms, m.p. 236° (*decomp.*) (Found: C, 51·16; H, 4·56; N, 17·69. $C_{17}H_{17}O_7N_5$ requires C, 50·62; H, 4·25; N, 17·36 per cent).

Similarly, from 5-methylindol-3-ylacetonitrile (1·8 g.) there was prepared *5-methyltryptamine* (0·85 g.) as a viscous yellow oil which solidified when allowed to stand over P_2O_5 at 20°/0·01 mm. for 16 hours. On washing the crude product (0·7 g.) with a 1 : 1 ether-*n*-hexane mixture, 5-methyltryptamine (0·55 g.), m.p. 93°–95°, was obtained. The monopicrate crystallised from ethanol in scarlet needles, m.p. 243° (*decomp.*) (Found: C, 51·00; H, 4·38; N, 17·29. $C_{17}H_{17}O_7N_5$ requires C, 50·62; H, 4·25; N, 17·36 per cent).

6-Methyltryptamine.—6-Methylindol-3-ylacetonitrile (1·5 g.) in ether (75 ml.) was reduced with lithium aluminium hydride (1 g.). After treating the complex with water and dissolving the alumina in sodium hydroxide (10N) the product, which was sparingly soluble in ether, was extracted with ethyl acetate and obtained by evaporation as a pale yellow solid (1·12 g.), m.p. 138°–139°. Recrystallization from benzene-*cyclo*hexane gave *6-methyltryptamine* as white needles, m.p. 141° (Found: N, 15·9. $C_{11}H_{14}N_2$ requires N, 16·07 per cent). Picrate, deep red needles from ethanol, m.p. 235° (*decomp.*) (Found: N, 17·2. $C_{17}H_{17}O_7N_5$ requires N, 17·36 per cent).

N-iso*Propyltryptamine.*—A solution of 1·3′-indolylethyl-2-bromide

(1 g.), m.p. 98°–99° [Hoshino and Shimodaira, 1935], in dry peroxide-free dioxan (10 ml.) was heated with *iso*propylamine (2 ml.) in a sealed tube at 100° for 8 hours. The cooled tube contained large crystals of *iso*propylamine hydrobromide and the contents were dissolved in hydrochloric acid (300 ml., 2N). The acid solution was repeatedly extracted with ether then the acid phase was made alkaline with sodium hydroxide (2N) to precipitate the crude product. By extraction with ethyl acetate and evaporation under reduced pressure, the tryptamine (0·8 g.) was obtained as a light brown solid. *N*-iso*Propyltryptamine* crystallized from *cyclo*hexane in needles, m.p. 99° (Found: C, 75·88; H, 8·96; N, 13·73. $C_{13}H_{18}N_2$ requires C, 77·09; H, 8·96; N, 13·85 per cent).

6-Benzyloxygramine.—Glacial acetic acid (2 ml.) was slowly added to dimethylamine (1·5 ml., 33 per cent aqueous solution), cooled in an ice-salt bath, and to the cold mixture formaldehyde (1 ml., 40 per cent solution) was added. The mixture was added to 6-benzyloxyindole (1·85 g.) and shaken until all solid dissolved. After standing overnight at room temperature the mixture was diluted with water (100 ml.), filtered, and the filtrate neutralized with sodium hydroxide (10N) to precipitate *6-benzyloxygramine* (2·7 g.), m.p. 136°–137° (from acetone) (Found: N, 10·1. $C_{18}H_{20}ON_2$ requires N, 10·0 per cent).

6-Benzyloxyindol-3-ylacetonitrile.—6-Benzyloxygramine (2 g.) dissolved in tetrahydrofuran (15 ml.) and glacial acetic acid (0·18 ml.) was added with shaking to a solution of dimethylsulphate (3·4 ml.) in tetrahydrofuran (6 ml.) and glacial acetic acid (0·18 ml.) at 10°–15° during 8 minutes. The mixture was allowed to stand in the dark at room temperature for 3 hours, when the methosulphate (2·5 g.) crystallized. The product was removed, washed with ether, dried, then heated with potassium cyanide (1·5 g.) in water (30 ml.) at 65°–70° for 1 hour. The mixture was cooled and extracted into ether, evaporation of which gave the nitrile (1·03 g.) as a pale yellow solid, m.p. 135°–137°. Recrystallization from 100° to 120° petroleum or aqueous acetic acid gave white needles of *6-benzyloxyindol-3-ylacetonitrile*, m.p. 137°–138° (Found: C, 77·7; H, 5·12; N, 10·48. $C_{17}H_{14}ON_2$ requires C, 77·8; H, 5·35; N, 10·7 per cent).

6-Benzyloxytryptamine.—The above nitrile (1 g.) was placed in a Soxhlet thimble and extracted with ether into a solution of lithium aluminium hydride (18 ml., approx. molar solution) in ether (25 ml.) for 3 hours then left overnight. The mixture was decomposed with water and sodium potassium tartrate solution (20 per cent), and the amine isolated from the ether phase as its insoluble hydrochloride (0·88 g.), m.p. 237°–238°, by shaking with 2N hydrochloric acid and filtering (Found: N, 9·36. $C_{17}H_{19}ON_2Cl$ requires N, 9·28 per cent). Some unchanged nitrile (0·1 g.) was recovered.

6-Hydroxytryptamine.—6-Benzyloxytryptamine hydrochloride (0·82 g.) was converted into its free base and hydrogenolysed in methanol

(30 ml.) with palladium-charcoal catalyst (0·2 g., 10 per cent Pd) until 70 ml. hydrogen were absorbed. After removing the catalyst, sulphuric acid (2·7 ml., N) was added to the methanol solution, and this was evaporated to give the tryptamine sulphate as a gum. The gum was dissolved in water (5 ml.) at 60°, creatinine (0·3 g.) and sulphuric acid (2·7 ml., N) added, the solution kept at 55°–60° for 10 minutes then diluted with hot acetone (70 ml.). After cooling to 0° (1 hour) the crystalline product was removed, dissolved in water and reprecipitated with acetone to give *6-hydroxytryptamine creatinine sulphate* (0·8 g.), m.p. 205°–210° (Found: C, 41·44; H, 5·57; N, 16·89. $C_{14}H_{23}O_7N_5S$ requires C, 41·47; H, 5·72; N, 17·28 per cent).

Amino-2:3-dialkyl Indoles.—These compounds were prepared by reduction of the corresponding nitro-compounds [Schofield and Theobald, 1950] in methanol with 10 per cent palladium-charcoal catalyst. *2-Methyl-3-ethyl-4-aminoindole*, m.p. 116°–118° (Found: C, 75·46; H, 8·0; N, 16·31. $C_{11}H_{14}N_2$ requires C, 75·84; H, 8·10; N, 16·08 per cent). *2-Methyl-3-ethyl-6-aminoindole*, m.p. 96°–97° (from petroleum, b.p. 80°–100°) (Found: C, 76·25; H, 7·83; N, 16·22 per cent). *2:3-Dimethyl-7-aminoindole hydrochloride*, m.p. > 300° (Found: Cl, 17·7; N, 14·0. $C_{10}H_{12}N_2HCl$ requires Cl, 18·03; N, 14·24 per cent).

Results

A series of indole compounds obtained from various commercial sources were tested both on the rat's uterus and the guinea-pig's ileum as antagonists of HT. The results are shown in Table I. Cinobufotenine (N:N:N-trimethyl-5-hydroxytryptamine) stimulated the guinea-pig's ileum in a concentration of 12·5 mg./l., being about 1/10 as active as HT. This effect was partly due to an action on tryptamine receptors, since it was diminished by specific tachyphylaxis in the presence of excess HT. It was also partly due to a nicotine action, since it was diminished by hexamethonium (10 mg./l.), which has no action against HT. It was antagonized by cocaine or atropine in much the same way that the effects of HT or nicotine are antagonized.

The rat's uterus was no more sensitive to cinobufotenine than the guinea-pig's ileum, but 6·5–50 mg./l. caused a marked contraction. When the drug had been washed out the effect of HT was scarcely affected, but the effect of acetylcholine was abolished. This surprising result has not been studied further.

Two of these compounds inhibited the response of the rat's uterus to HT without affecting the response to a choline ester, and their inhibitory action on this tissue was therefore classified as specific. The inhibition caused by the other compounds was unspecific. In experiments on guinea-pig's ileum histamine was used as a control substance and all the observed antagonisms were judged to be unspecific. Since

the effect of gramine on the rat's uterus appeared specific, a large number of derivatives of gramine were made and tested on this tissue.

Most of the other compounds were tested as follows. A dose of 10–15 ng.[1] of HT (calculated as base) generally caused a suitable contraction of the rat's uterus when added to the bath (2 ml.). Some such dose was added at regular intervals (3–5 min.) until a regular response

TABLE I.—PRELIMINARY TESTS

Lowest concentrations (mg./l.) observed to cause stimulation (S), partial inhibition (PI) and complete inhibition (CI) of the responses to HT or T. Sat. = 0·1 ml. of a saturated solution in 2 ml. bath.

Compound	Rat's uterus			Guinea-pig's ileum		
	S	PI	CI	S	PI	CI
A. Effect on rat's uterus specific						
Gramine	5	..	5	0·5	0·5	50
2-Methylindole	sat.	..	sat.	sat.	..	sat.
B. Effects unspecific						
Cinobufotenine	0·25	50	..	12·5	..	12·5
Indole	5	5	25	0·5	0·5	50
3-Methylindole	sat.	sat.	sat.	sat.	sat.	sat.
7-Methylindole	sat.	..	sat.	sat.	sat.	..
2-Methyl-3-acetylindole	sat.	..	sat.	..	..	..
3-Indolylacetic acid	25	10	25	0·5	5	50
Methyl 3-indolylacetate	5	5	25	5	25	25
3-Indolylpropionic acid	25	25	..	5	5	50
3-Indolylbutyric acid	sat.	sat.	..	sat.	sat.	..
2 : 3-Indolinedione (Isatin)	50	50	50	50	50	50
Isatin-β-oxime	sat.	sat.	sat.	sat.	sat.	sat.
dl-Tryptophan	25	25	..	25	25	..
Hypaphorine hydrochloride	6·25	25	..	50	50	..
Adrenochrome	0·5	0·05	0·5	50	0·5	50
Adrenolutin	sat.	sat.	..	sat.	sat.	sat.

was obtained. The dose was then altered and a curve constructed by plotting the height of the response against the dose. The drugs to be tested as antagonists were added in an initial concentration of 10^{-6} (1 μg./ml.) and HT was reapplied. The concentration of the antagonist was maintained in the bath for 60 min., either by adding fresh doses whenever the fluid was changed, or by adding the drug to one of the reservoirs from which the bath could be refilled. If the effect of HT was not definitely diminished, higher concentrations of the potential antagonists were tried. When the effect of HT was diminished, the dose of HT was increased, until the response was 50 per cent of the maximum response at the beginning of the experiment, or until it became apparent that this result could not be achieved.

The Dose-Ratio.—The effects of active and specific antagonists were calculated in terms of the dose-ratio (A/A_0) by comparing effects in the

[1] 1 ng. = 1 nanogram = 0·001 μg.

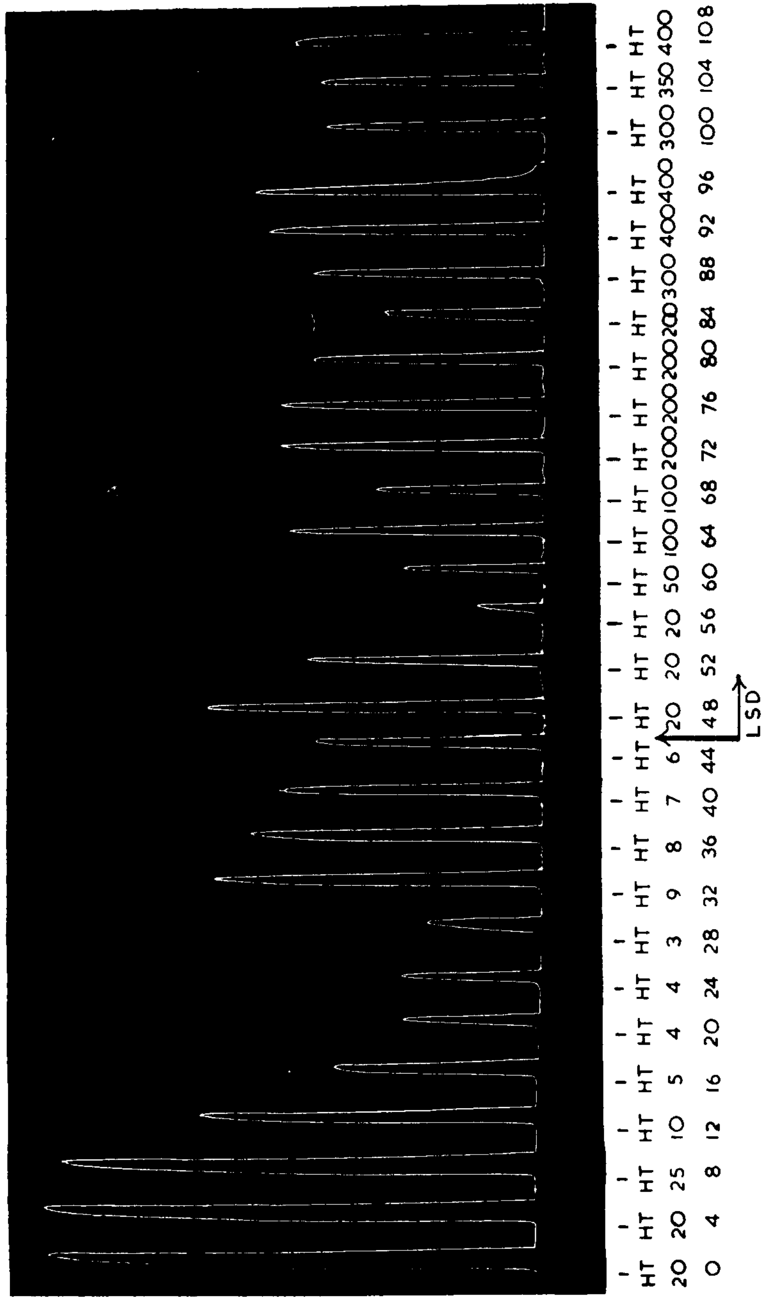


Fig. 1.—Rat's uterus. 2 ml. bath. Measurement of dose-ratio. HT: 5-Hydroxytryptamine (ng.). LSD: Lysergic acid diethylamide (10 μg./l.) added at 45 min. and then maintained.

presence of the antagonist with the initial dose-effect curve. Fig. 1 shows an experiment of this kind with lysergic acid diethylamide. After the dose-effect curve of HT had been plotted, lysergic acid diethylamide (LSD) in a concentration of 10 μg./l. was added to the bath at 45 min., and this concentration was maintained in the bath for 60 min. or more. After 3 min. contact with LSD, a dose of 20 ng. of HT produced an effect equal to that caused by 10 ng. of HT at the beginning of the experiment. The dose-ratio (A/A_0), therefore, was 20/10 after 3 min. After 15 min., a dose of 50 ng. of HT produced an effect similar to that

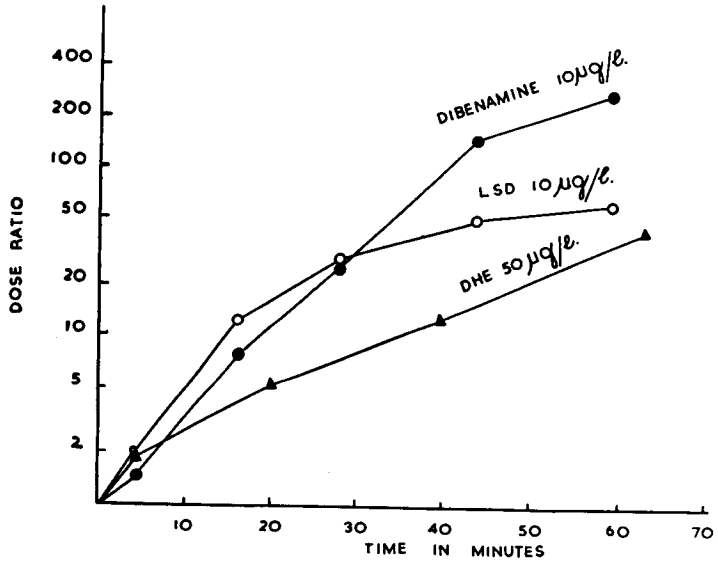


FIG. 2.—Relation between dose-ratio (log scale) and time. DHE: Dihydroergotamine. LSD: Lysergic acid diethylamide.

caused by 4 ng. of HT in the absence of LSD and the dose-ratio became 50/4. This ratio went on increasing with time, and at 72 min. it was 200/7, at 88 min. 300/6 and at 104 min. 350/6. Dose-effect curves were constructed and used to interpret the results when necessary.

The dose-ratio was then plotted against time and the curves obtained for various concentrations of the different compounds (figs. 2 and 3). Such curves were constructed only for those compounds which gave a dose-ratio of 10 or higher, and had a specific anti-HT action. The shapes of the curves obtained in this way may vary considerably. The three compounds shown in fig. 2 were the most active compounds tested when judged by their effects after 60 min., but these effects developed slowly, and after 10 min. these compounds showed little effect in the

low concentrations used in these experiments. The effect of dihydroergotamine was particularly slow to develop.

In the low concentrations (10 μg./l.) of LSD and dibenamine shown in fig. 2, the dose-ratio appeared to be approaching a steady value after 60 min., but with higher concentrations of these drugs the effects went on increasing indefinitely. The effect of dihydroergotamine in a concentration of 50 μg./l. as shown in fig. 2 was still increasing even after 60 min. In a concentration of 10 μg./l. dihydroergotamine did not give a high dose-ratio, but lysergic acid diethylamide and dibenamine were very active at this dose level.

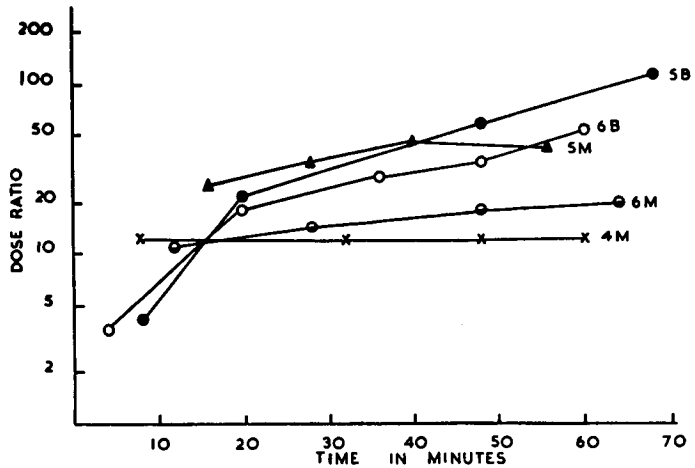


FIG. 3.—Relation between dose-ratio (log scale) and time. Concentrations 1 mg./l. 5B and 6B: 5- and 6-Benzyloxygramine. 4M, 5M and 6M: 4- 5- and 6-Methylgramine.

The effects of methylgramines were complete earlier (fig. 3). With 1-, 5- and 6-methylgramines the state of equilibrium was established after 30–40 min. The effect of 4-methylgramine, however, was complete within 10 min. 1-Methylgramine did not show much activity in the concentration used (1 mg./l.). The dose-ratio with this compound rose to the maximum figure of 6·6.

The effects of most of the compounds were complete within 60 min. in the concentrations used in the experiments. Sometimes, with the most active compounds, even after 60 min. it was impossible to be sure that the effect was complete; but long times introduce errors of their own, and 60 min. was adopted as the standard time at which measurements were made.

It has already been pointed out that the dose-ratio has especial value when it is independent of the dose of the agonist (HT), and this appears

to be the case with some at least of the drugs considered here. Fig. 4 shows log-dose-effect curves for HT in the presence and absence of 6-methylgramine. The fact that the horizontal distance between these curves is approximately constant shows that the dose-ratio was approximately constant. Similar results have been obtained with all the last eleven drugs in Table II.

It has not been possible to do similar experiments with some of the more active compounds, such as dihydroergotamine, because the dose-ratio continues to increase almost indefinitely. When the same concentrations of a given drug were used more than once, the values obtained for the dose-ratio at given times appeared to be fairly constant.

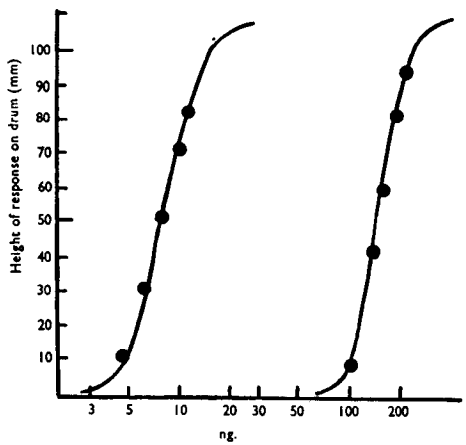


FIG. 4.—Vertical scale—response of rat's uterus in 2 ml. bath. Horizontal scale—dose of HT (log scale). On the left before, on the right after, the addition of 6-methylgramine (1 mg./l.).

The Drug-Ratio.—The drugs shown in fig. 3 can be compared with one another by measuring the effect produced after 60 min. This method can, however, only be used when a concentration is found in which all the drugs have some effect. Some of the drugs in the present series had no effect at all in a concentration of 10^{-6}, but were effective in higher concentrations; others were effective in lower concentrations. The opinion has already been expressed that the most satisfactory general index of the activity of antagonists is the pA_x, but quicker results may be obtained by estimating the drug-ratio, which is equal to the ratio of the concentration of the agonist to the concentration of the antagonist (A/B) when the response is 50 per cent of the initial maximum response. This can be estimated from the results of a single experiment of the type described above. It is unlikely to give reliable results when the

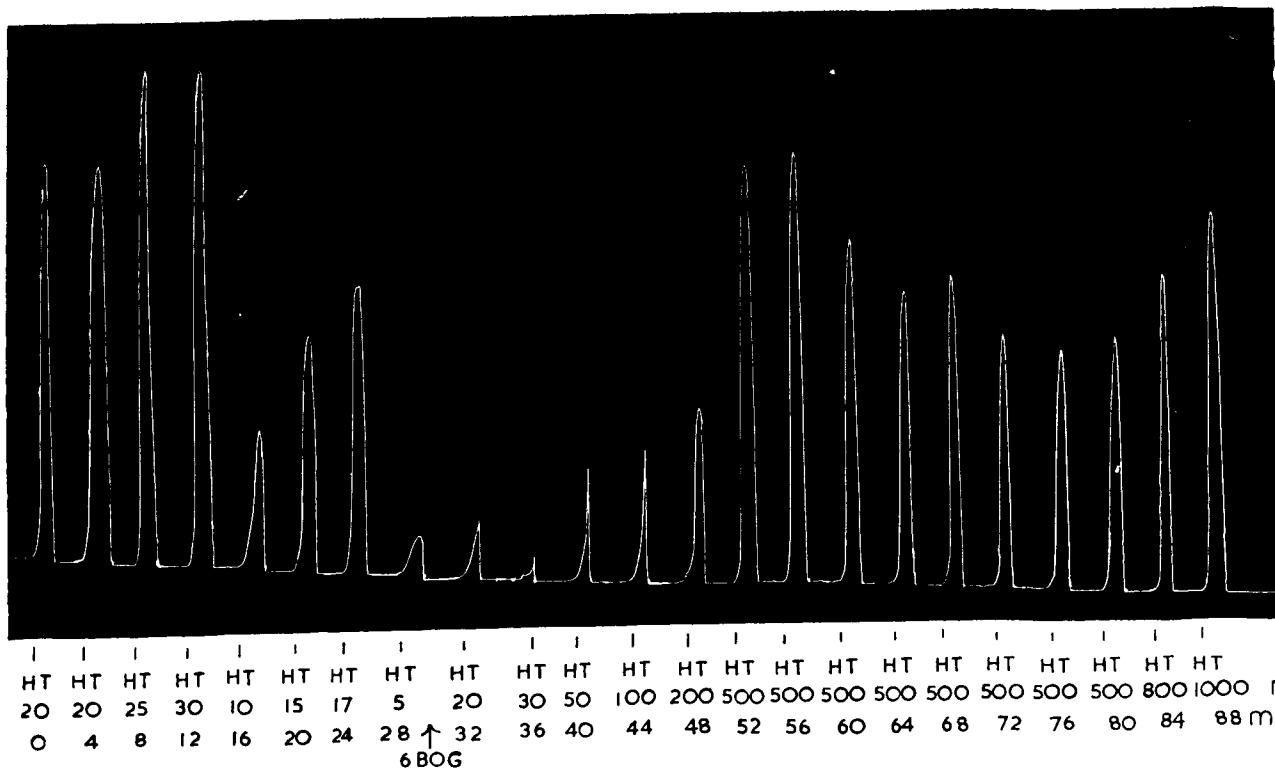


FIG. 5.—Rat's uterus. 2 ml. bath. Measurement of drug ratio. HT: 5-Hydroxytryptamine (ng.). 6 BOG: 6-benzyloxygramine (1 mg. l.) added at 29 min. and then maintained.

dose-ratio (A/A_0) is small, and the concentrations were therefore always high enough to produce a dose-ratio of at least 5 and generally much more.

This method of calculation, which has often been used before, is convenient, because it can be used to compare results when different concentrations of antagonists are used, as is necessary when their activities vary widely. It is more convenient than the pA_2 in preliminary screening tests, because an estimate can often be obtained in a single experiment using only one concentration of the antagonist. Its theoretical basis will be discussed later.

Fig. 5 illustrates one such experiment, and it will be seen that the design is similar to that used for the estimation of the dose-ratio (see fig. 1), except that it is unnecessary to determine the shape of the dose-effect curve at the beginning of the experiment. Fig. 5 shows that 30 ng. of HT caused a maximal contraction. From the time 29 min., 6-benzyloxygramine (1 mg./l.) was continuously present in the bath. It caused a marked inhibition of the effect of HT, but the inhibition was surmounted by increasing the dose. The doses were continuously adjusted with the object of obtaining 50 per cent of the original maximal response. At 72 min. a dose of 500 ng. of HT in the 2 ml. bath produced a response equal to about 50 per cent. of the original maximum, and thereafter this response was about constant. The drug-ratio after 43 min. was therefore 250/1000 or 1/4. When the drug-ratio is less than 1, it is given as a fraction, in the belief that this is easier to remember than a decimal.

The results shown in Tables II and III were obtained in this way. The drugs listed in Table II had a specific antagonistic effect to HT, in the sense that the concentrations of those drugs used had no effect on

TABLE II.—COMPARATIVE POTENCIES (DRUG-RATIOS) OF SPECIFIC ANTAGONISTS

Names of the compounds	Drug-ratio
Dibenamine hydrochloride	35–50
Lysergic acid diethylamide (LSD)	20
Dihydroergotamine methane sulphonate	4–5
Dihydroergocornine methane sulphonate	1·25
Dihydroergokryptine methane sulphonate	1/2–1/1·3
5-Benzyloxygramine	1/2·4
6-Benzyloxygramine	1/4
5-Methylgramine	1/10
6-Methylgramine	1/16
4-Methylgramine	1/28
N : N-Dimethyltryptamine	1/40
aa-Dimethyltryptamine	1/72
1-Methylgramine	1/80
2 : 3-Dimethyl-6-aminoindole	1/84
a-Ethyltryptamine	1/200
6-Amino-1 : 2 : 3 : 4-tetrahydrocarbazole	1/220
2-Methyltryptamine	1/400
2-Methyl-3-ethyl-5-aminoindole	1/660

TABLE III.—COMPARATIVE POTENCIES (DRUG-RATIOS) OF UNSPECIFIC ANTAGONISTS

Names of compounds	Drug-ratio	Recovery
A. No alcohol		
N-Ethyl-N-(1-naphthylmethyl)-2-bromoethylamine (SY–28)	2	Partial or none
Dihydroergocristine methane sulphonate	1·75	Partial or none
Mepyramine maleate	1/40	Slow
7-Amino-1 : 2 : 3 : 4-tetrahydrocarbazole	1/66	Slow
Diphenhydramine	1/80	Slow
Atropine sulphate	1/200	Slow
8-Amino-1 : 2 : 3 : 4-tetrahydrocarbazole	1/800	Slow
2-Methyl-3-ethyl-6-aminoindole	*	Partial
5-Hydroxyindole	0	..
6-Hydroxyindole	0	..
Tryptophol (3-indolylethyl alcohol)	0	..
B. Final concentration of alcohol < 0·25 per cent		
2-Methyl-3-ethyl-7-nitroindole	1/2	Partial
5-Nitro-1 : 2 : 3 : 4-tetrahydrocarbazole	1/10	Quick
4-Nitrocarbazole	1/12	Slow
2-Nitrocarbazole	1/26	Slow
1-(3-Indolyl)-2-methyl-2-nitropropane	1/26	Slow
2-Methyl-3-ethyl-4-nitroindole	1/30	Partial
7-Nitro-1 : 2 : 3 : 4-tetrahydrocarbazole	1/40	Quick
1-(3-Indolyl)-2-nitrobutane	1/60	Slow
3-Nitrocarbazole	1/80	Slow
1-Nitrocarbazole	1/100	Slow
1-(3-Indolyl)-2-bromoethane	1/200	Slow
1-Methylindole	1/250	Slow
2-Acetylindole oxime	1/400	Slow
5-Methylindol-3-ylacetonitrile	1/420	Quick
4-Methylindole	1/660	Slow
7-Methylindol-3-ylacetonitrile	1/800	Quick
4-Methylindol-3-ylacetonitrile	1/1000	Quick
2-Methylindol-3-ylacetonitrile	1/1330	Quick
C. Final concentration of alcohol > 0·25 per cent		
2 : 3-Dimethyl-6-nitroindole	1/22	Partial
2-Phenyl-3 : 5-dinitroindole	1/40	Partial
6-Nitro-1 : 2 : 3 : 4-tetrahydrocarbazole	1/90	Quick
2 : 3-Dimethyl-4-nitroindole	1/150	Partial
2-Methyl-3-ethyl-7-aminoindole	1/200	Slow but complete
6-Methylindole	1/250	Slow
5-Methylindole	1/1000	Slow
3-Indolyl-acetonitrile	1/1000	Slow
2-Methyl-3-carbethoxy-5-methoxyindole	*	Quick
1-Methylindol-3-ylacetonitrile	*	Quick
2-Methyl-3-ethyl-5-nitroindole	*	Partial
2-Methyl-3-ethyl-6-nitroindole	*	Partial
8-Nitro-1 : 2 : 3 : 4-tetrahydrocarbazole	*	Quick

* Inactive in the ordinary concentrations (1–10 mg. l.). Higher concentrations unsurmountable.

the response to acetylcholine. The effects of the drugs listed in Table III were classified as unspecific because the effect of acetylcholine was depressed, although usually less than the effect of HT. The classification is thus somewhat arbitrary.

It so happens that the specific antagonists in Table II were all soluble in acid saline and that ethanol had to be used to dissolve many of the unspecific drugs in Table III. It was found that the presence of 0·25 per cent of ethanol in the bath was just sufficient to have a direct inhibitory action on the response to HT. When ethanol was used to dissolve the drug the final concentration of this substance in the bath was therefore calculated and the results are subdivided accordingly in Table III. In the last group of drugs (C) the amount of alcohol was sufficient to have some direct effect, and the activity of the drug was probably less than the estimate given; it cannot have been more.

TABLE IV.—DRUG-RATIOS AT DIFFERENT CONCENTRATIONS

Names of the compounds	Concentrations μg./l.	Drug-ratio $\left(\frac{\text{conc. of HT}}{\text{conc. of antagonist}}\right)$ 1 hour
Dibenamine hydrochloride	1/2	24–30
	10	35–50
	100*	> 500
Lysergic acid diethylamide	1/2	16
	10	20
	100	> 150
Dihydroergotamine methanesulphonate	10	5
	50	4–5
	100	> 100
5-Benzyloxygramine	100	1/3·2
	1000	1/2·5
	5000*	> 2
5-Methylgramine	200	1/7·4
	500	1/8·4–1/7·2
	1000	1/10
	3000*	1/17–1/15

* With these concentrations, the response to acetylcholine was also depressed and the effect therefore was unspecific.

The drug-ratio has especial value when it is independent of the doses of both drugs. Table IV shows estimates of the drug-ratio at various concentrations of the antagonists. When low concentrations were used, the drug-ratios were more or less constant over a fairly wide range, but with high concentrations of most of these active drugs, the drug-ratio increased suddenly, so that HT caused less than 50 per cent of the maximum response even in the largest doses which could be tested.

One of these drugs however (5-methylgramine) differed from the others, since the drug-ratio showed no tendency to rise, but fell slightly, and higher concentrations of this drug actually stimulated the uterus.

The pA_x.—Some of the more potent drugs were then studied in more detail and estimates made of the pA_2 by a method rather different from that used by Schild [1947]. This is illustrated in figs. 6 and 7. A dose of HT was found which caused 50 per cent of the maximum effect, and double this dose was then applied at various times after the addition of

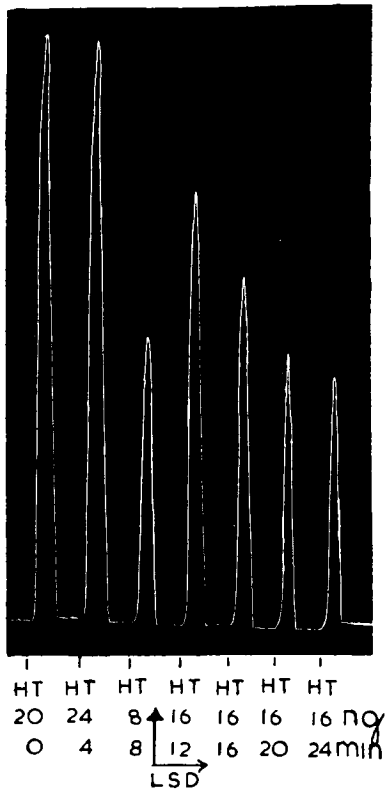


FIG. 6.—Rat's uterus. 2 ml. bath. Measurement of pA_2 (9 min.). HT: 5-Hydroxytryptamine (ng.). LSD: Lysergic acid diethylamide added at 9 min. and then maintained.

the antagonist, the concentration of which was kept constant. In the experiment shown in fig. 6, 8 ng. of HT caused half the maximum effect. LSD (1 μg./l.) was then added to the bath at time 9 min., and the double dose of HT (16 ng.) applied at intervals. The time at which the effect of this double dose was equal to the original effect of the single dose was between 16 and 20 min. and was taken as 18 min., or 9 min. after the addition of the antagonist. The pA_2 (9 min.) for LSD was thus estimated as 8·5, which is equal to the negative logarithm of the molar concentration corresponding to 1 μg./l. In some cases the experiment

can then be continued using larger doses of the agonist to measure the pA_{10}, etc.

Such results cannot be used directly to compare one drug with another because the time corresponding to each estimate varies uncontrollably. When, however, a series of different concentrations of the antagonist has been used, the relation between pA_x and time can be plotted as shown in fig. 7. It will be seen that the pA_2 of the 4 drugs

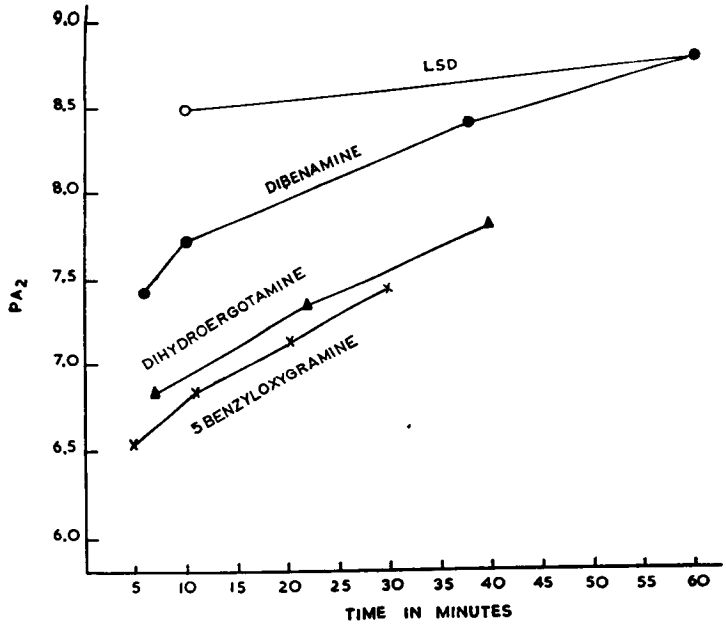


FIG. 7.—Relation of pA_2 to time.

shown here continued to increase for an hour or so. These curves are similar to, but not quite the same as, those obtained by estimating the dose-ratio and shown in fig. 2. In comparing these two figures it should be remembered that the concentrations in fig. 2 are calculated directly and that those in fig. 7 are calculated on a molar basis. This does not, however, account for the main differences, which are presumably due to the fact that fig. 7 shows the times to reach a dose-ratio of 2 and fig. 2 shows the times for larger effects.

Table V shows a comparison between estimates of the pA_2 of various drugs at 10 min. The values for the last 3 drugs in this table became steady in less than 10 min.

Stimulation followed by Depression of the Receptors.—The 12 indole compounds shown in Table VI caused stimulation of the uterus, but this was followed by a specific depression of the tryptamine receptors.

These drugs were kept in the bath for 10 min. in a concentration of 10 mg./l. or more and then washed out. When the muscle had relaxed, it gave a diminished response to HT and a normal response to acetylcholine. The response to HT then gradually recovered. These compounds thus behaved like HT itself, which also causes stimulation

TABLE V.—RESULTS OF pA_2 DETERMINATIONS

Names of the compounds	pA_2 after 10 min. contact
Lysergic acid diethylamide (LSD)	8·5
Dibenamine	7·72
Dihydroergotamine	6·9
Dihydroergokryptine	6·82
5-Benzyloxygramine	6·79
5-Methylgramine	6·57
Mepyramine	6·3 (6 min.)
Diphenhydramine	5·86
Atropine	5·84 (4 min.)

TABLE VI.—COMPOUNDS CAUSING STIMULATION OF THE UTERUS FOLLOWED BY SPECIFIC INHIBITION

Names of the compounds	Conc. in the bath for 10 min. to inhibit HT, mg./l.
Bufotenine (N : N-dimethyl-5-hydroxytryptamine) creatinine sulphate	10
Gramine methosulphate	50–100
2-Methylgramine	50
5-Hydroxygramine	5–10
6-Hydroxytryptamine creatinine sulphate	100
1-Methyltryptamine	10
4-Methyltryptamine	10
5-Methyltryptamine	10
6-Methyltryptamine	10
7-Methyltryptamine	1–10
α-Methyltryptamine	10
N-Isopropyltryptamine	10

followed by depression of the tryptamine receptors [Gaddum, 1953 *a*; Gaddum and Hameed, 1954). The experiments with the first three of these drugs were difficult to reproduce, because the contractions continued for some time when the drugs were washed out, but clear evidence was eventually obtained.

The initial stimulant actions of these compounds were comparatively feeble, and in most cases the effective dose was 500–1500 times the effective dose of HT. Bufotenine was the most active (1/15–1/40 HT) and 6-hydroxytryptamine was the least active (1/10,000 HT).

The stimulant effects of most of these compounds were probably due to an action on the tryptamine receptors, since they were antagonized specifically by drugs such as 5-benzyloxygramine, 5-methylgramine and 4-methylgramine. Subthreshold concentrations sometimes increased the response to HT.

Most of the compounds listed in Table VII caused stimulation of the uterus followed by depression of the responses to both HT and acetylcholine. Tetrahydroharman and 2- and 3-aminocarbazole in a concentration of 10 mg./l. caused stimulation, not followed by inhibition. Higher concentrations (25 mg./l.) caused no stimulation but marked unspecific inhibition.

TABLE VII.—COMPOUNDS CAUSING STIMULATION OF THE UTERUS FOLLOWED BY UNSPECIFIC INHIBITION

Names of the compounds	Conc. which caused stimulation, mg./l.
2-Methyl-3-ethyl-4-aminoindole	50
2 : 3-Dimethyl-4-aminoindole	50
2 : 3-Dimethyl-7-aminoindole	25
2 : 3-Dimethyl-7-nitroindole	1
3-Carbethoxy-6-nitroindole	1
1-Aminocarbazole	10
2-Aminocarbazole	10
3-Aminocarbazole	10
5-Amino-1 : 2 : 3 : 4-tetrahydrocarbazole	25
Tetrahydroharman	10

Reversibility.—The recovery of the response to HT when the antagonists were removed from the bath was observed for at least 1 hour in most of the experiments described above. The effects of dibenamine and the various ergot derivatives were partially reversible when the concentration was below 10 μg./l., but became irreversible when it was higher than this. The effects of the benzyloxygramines and the methylgramines were also reversible after low concentrations (10 μg./l.), but became partially or completely irreversible when the concentration was 1 mg./l. or more. When the various tryptamine derivatives were removed from the bath recovery was slow, but after 2-methyl-3-ethyl-5-aminoindole it was rapid.

The reversibility of the effects of various other drugs are shown in Table III.

DISCUSSION

Various attempts have been made to calculate a single figure which would express the activity of an antagonist over a wide range of concentrations and effects, but this is only possible when the results are governed by known laws. Sometimes the antagonism appears to be

governed by the mass laws [Clark, 1937; Gaddum, 1937, 1943; van Maanen, 1950; Chen and Russell, 1950].

In their simplest form these laws have led to the following conclusions.

1. The relation between y (the effect calculated as a proportion of its maximum value) and the concentration of the agonist (A_1) should be given by the equation $KA = y/(1-y)$ [Clark, 1937]. When this is so, the log-dose–effect curve is sigmoid and symmetrical and may be converted into a straight line by converting y into logits [Berkson, 1944] and plotting these against log dose. It is generally more convenient to use probits, which are practically the same as logits, and when this is done the slope of the regression line (b) should be about 1·4. Log-dose-effect curves for HT and the rat's uterus were found to be symmetrical but steep; when the slope was calculated by this method it was in the range (4) 2·5–4·5. Chen and Russell [1950] plot $1/y$ against $1/A$ as recommended by Lineweaver and Burk [1934], and this gives straight lines when $KA = y/(1-y)$. When the slope with probits is greater than 1·4, the lines obtained by this method of plotting should be concave upwards, and this was actually the case with our data.

2. The log-dose–effect curves with and without the antagonist should be parallel, or, in other words, the dose-ratio should be independent of the response. This was so in every case tested (fig. 4).

3. The response (y) should be constant, so long as $(A - A_0)/B$ is constant, where B is the concentration of the antagonist. In our experiments A was generally large compared with A_0, so that this expression was practically equal to A/B—the drug-ratio. This quantity provides a convenient measure of the potency of an antagonist. It has often been used for this purpose, and its use is justified by the facts the mass laws predict that it should be constant over a wide range of doses and that this is often so.

In our experiments the drug-ratio was nearly constant over a certain range of doses, but liable to increase when high concentrations of active antagonists were used (Table IV).

Similar phenomena have been observed before and explained in different ways.

1. Nickerson [1949] found that dibenamine produced "non-equilibrium" block which could not be overcome by large doses of adrenaline, and attributed this to the fact that the effect of the dibenamine on the receptor was irreversible.

2. Various workers have found that certain antagonists diminish the slope of the log-dose–effect curve and the height of the maximum response to large doses [Schild, 1949]. When this maximum is diminished by more than 50 per cent the drug-ratio must become infinite. Such results have been explained on the theory that, although the actions of both drugs are reversible, the antagonism is "non-competitive" in the

sense that the antagonistic drug inhibits receptors which are already occupied by the active drug just as readily as those which are not [Chen and Russell, 1950; Schild, 1954]. Chen and Russell concluded that low concentrations of the drug SY80 (which resembles dibenamine; see Table III) antagonized adrenaline competitively, but when higher concentrations were used their graphs showed that the maximum response to large doses was diminished, and they concluded that the antagonism was non-competitive. Their results agree with ours in showing that low concentrations of this type of drug act like a competitive antagonist, and that higher concentrations have more effect than would be expected.

3. If the antagonist eventually inhibits a late stage in the metabolic pathway leading from the receptor to the contraction of the muscle, high concentrations of the stimulant drug should not be more effective than that which just causes maximal stimulation of one of the earlier stages of this pathway; this may well cause less than half of the original maximum effect.

In the absence of precise knowledge of the mechanisms involved it is best to use words which do not depend on any one theory, and it is for this reason that the word unsurmountable is used to describe antagonisms which cannot be overcome by increasing the concentration of the active drug.

The Comparison of the Different Drugs

The estimation of potencies after 1 hour might be criticized because of a possible change of sensitivity of the preparation after such a long interval. Experiments without antagonists gave no evidence of changes in sensitivity in this time and control injections of acetylcholine had constant effects, except when the antagonism was classified as unspecific. Repeated estimates of the pA_2 gave consistent results.

The specific antagonists studied can be divided into two classes:

(1) The more potent drugs (such as dibenamine, LSD and the benzyloxygramines) acted slowly and eventually produced irreversible and unsurmountable effects.

(2) The less potent drugs (such as the methylgramines, tryptamines, 2-methyl-3-ethyl-5-aminoindole, etc.) acted more quickly and their actions were more easily reversible.

Structure and Action

It must be emphasized that all these results apply only to the rat's uterus.

Methylindoles.—Indoles with methyl groups in positions 1, 4, 5, 6 and 7 have all been tested and found to have feeble unspecific depressant

actions on the uterus. The antagonistic action of 2-methylindole was also feeble, but appeared to be specific (Table I).

Methyl-3-indolylacetonitriles.—These compounds were also feeble and unspecific antagonists, but acted quickly and were quickly washed out. The descending order of activities for different positions of the methyl group was 5, 7, 4, 1.

Aminoindoles.—2-Methyl-3-ethyl-5-aminoindole and 2:3-dimethyl-5-aminoindole were found by Woolley and Shaw [1953] to antagonize the action of HT on the carotid artery. These compounds have a similar action on rat's uterus, and this action is specific, though not very powerful. We have not yet tested the more potent compounds described by Shaw and Woolley [1954]. Similar compounds with an amino group in position 4 or 6 had weak unspecific depressant actions. The corresponding nitro compounds were also inactive.

Gramine Derivatives.—Gramine itself has already been shown to be a specific antagonist for HT [Gaddum and Hameed, 1954], and some of its derivatives have now been found to be much more active. Four of the monomethylgramines depressed the response to HT quickly and specifically in low concentrations without causing a contraction, and the descending order of activities for different positions of the methyl group was 5, 6, 4, 1.

In low concentrations 2-methylgramine and gramine methosulphate had the opposite effect and made the muscle more sensitive to HT. Higher concentrations of these two drugs caused a contraction followed by specific inhibition (Table VI).

The most active of the new synthetic indole compounds were 5- and 6-benzyloxygramine, and these caused a specific inhibition which developed slowly.

Unlike most of the other gramine derivatives 5-hydroxygramine caused contraction of the uterus.

Tryptamines.—Substituted tryptamines with a methyl group in position 1, 4, 5, 6 or 7 all stimulated the uterus before causing specific inhibition. The substitution of a methyl group in position 2 had the opposite effect to that seen among the gramine derivatives, since it led to the formation of a specific antagonist with no initial stimulant action.

N : N-Dimethyltryptamine was a much more potent antagonist than gramine. If its activity can be increased, like that of gramine, by substituting a methyl or benzyloxy group in position 5, the resulting compound should be very potent, but these compounds are not yet available. α-Methyltryptamine caused contraction, but $\alpha\alpha$-dimethyltryptamine and β-ethyltryptamine did not. The explanation of these observations remains speculative.

It is interesting that 6-hydroxytryptamine has a very feeble action like that of HT, being about 10,000 times less active.

Carbazole Derivatives.—A number of amino- and nitro-derivatives of carbazole have been tested, but only one of these (6-amino-1 : 2 : 3 : 4-tetrahydrocarbazole) had a specific inhibitory effect, and that was feeble.

Lysergic Acid Derivatives.—Lysergic acid diethylamide was the most active compound, and the closely allied ergometrine (lysergic acid propanolamide) had no action. Dihydroergotamine was more active than ergotamine against HT, as it is against adrenaline [Rothlin, 1944]. The potencies of dihydroergotamine and dihydroergocristine were greater than those of dihydroergocornine and dihydroergokryptine, and this may be due to the presence of phenylalanine in the molecule. On the other hand, the effect of dihydroergocristine was unspecific, and it was also found that dihydroergocornine had a narrow range of specificity. Both these drugs contain dimethylpyruvic acid, and it seems possible that this part of the molecule is associated with antagonism to acetylcholine.

It has been suggested [Gaddum, 1953 *b*; Woolley and Shaw, 1954; Amin, Crawford and Gaddum, 1954] that the effects of LSD on the brain may be due to interference with a physiological mechanism depending upon HT. Hoffer, Osmond and Smythies [1954] have found that adrenochrome may also have curious effects upon the brain, and since adrenochrome is an indole compound which can easily be formed from adrenaline *in vitro*, they have suggested that the action of adrenochrome on the brain may be similar to that of LSD, and that mental disorders may be due to the formation of this substance in the body. If adrenochrome had been found to be a potent antagonist of HT on the rat's uterus, this theory would have been supported. Actually it only had an unspecific action, which was probably due to the presence of 0·17 per cent of adrenaline in the sample used.

Summary

1. A number of synthetic indole compounds have been tested as antagonists for 5-hydroxytryptamine (HT) in experiments on rat's uterus.

2. Antagonisms are classified as unspecific except when it has been possible to inhibit the response to HT without altering the response to acetylcholine.

3. The time course of the antagonisms has been followed by estimating the dose-ratio: that is, the ratio of equiactive doses of HT before and after the administration of the antagonist.

4. The activity of different compounds has been compared by estimating the drug-ratio, which is equal to the ratio of the dose of HT to the dose of antagonist, when the effect after 1 hour is 50 per cent of the original maximal effect. This gives results in general agreement with estimates of the pA_2, and is more convenient when there are many substances to be tested.

5. The effects of the more active compounds, such as dibenamine, lysergic acid diethylamide and benzyloxygramine, developed slowly and became irreversible. Eventually the blockade could not be overcome even by enormous doses of HT, and it is then said to be unsurmountable.

6. The effects of less active specific antagonists (such as methylgramines) developed more rapidly, and were reversible and surmountable.

REFERENCES

Amin, A. H., Crawford, T. B. B. and Gaddum, J. H. (1954). *J. Physiol.* [In the press.]

Beraldo, W. T. and Rocha e Silva, M. (1949). *J. Pharmacol.* **97,** 388.

Berkson, J. (1944). *J. Amer. statist. Ass.* **39,** 357.

Chen, G. and Russell, D. (1950). *J. Pharmacol.* **99,** 401.

Clark, A. J. (1937). General Pharmacology. Heffter's Handbuch.

Erspamer, V. (1952). *Ricerca Sci.* **22,** 694, 1568, 2148.

Fleckenstein, A. (1952). *Brit. J. Pharmacol.* **7,** 553.

Gaddum, J. H. (1926). *J. Physiol.* **61,** 141.

Gaddum, J. H. (1937). *J. Physiol.* **89,** 7 *P.*

Gaddum, J. H. (1943). *Trans. Faraday Soc.* **39,** 323.

Gaddum, J. H. (1953 *a*). *J. Physiol.* **119,** 363.

Gaddum, J. H. (1953 *b*). *J. Physiol.* **121,** 15 *P.*

Gaddum, J. H. and Hameed, Khan A. (1954). *Brit. J. Pharmacol.* **9,** 240.

Hoffer, A., Osmond, H. and Smythies, J. (1954). *J. ment. Sci.* **100,** 29.

Hoshino, T. and Shimodaira, K. (1935). *Liebigs Ann.* **520,** 19.

Lineweaver, H. and Burk, D. (1934). *J. Amer. chem. Soc.* **56,** 658.

van Maanen, E. F. (1950). *J. Pharmacol.* **99,** 255.

Nickerson, M. (1949). *Pharmacol. Rev.* **1,** 27.

Reuse, J. J. (1948). *Brit. J. Pharmacol.* **3,** 174.

Rocha e Silva, M. (1950). *Exp. Med. Surg.* **8,** 346.

Rocha e Silva, M. and Beraldo, W. T. (1948). *J. Pharmacol.* **93,** 457.

Rothlin, E. (1944). *Helv. physiol. Acta,* **2,** 48.

Rydon, H. N. (1948). *J. chem. Soc.* 705.

Schild, H. O. (1947). *Brit. J. Pharmacol.* **2,** 189.

Schild, H. O. (1949). *Brit. J. Pharmacol.* **4,** 277.

Schild, H. O. (1954). *J. Physiol.* **124,** 33 *P.*

Schofield, K. and Theobald, R. S. (1950). *J. chem. Soc.* 1505.

Schöpf, C. and Thesing, J. (1951). *Angew. Chem.* **63,** 377.

Shaw, E. and Woolley, D. W. (1954). *J. Pharmacol.* **111,** 43.

Supniewski, J. V. and Scrafin-Gajewski, M. (1940). *Chem. Abstr.* **34,** 6410.

Thesing, J. and Schülde, F. (1952). *Ber. dtsch. chem. Ges.* **85,** 324.

Woolley, D. W. and Shaw, E. (1953). *J. Amer. chem. Soc.* **75,** 1877.

Woolley, D. W. and Shaw, E. (1954). *Brit. med. J.* ii, 122.