Chapter 3

Bioassay of indolealkylamines*

By

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With 5 Figures

I. Extraction of 5-HT and related indolealkylamines from tissues and organic fluids

For a satisfactory extraction of 5-HT from tissues it is sufficient that they be carefully cleaned, minced finely with scissors and then treated with 4 parts (w/v) of pure acetone, to give a final concentration of acetone of approximately 80%.

The completeness and accuracy of this extremely simple procedure, first suggested by ERSPAMER (1940), has been generally confirmed (FELDBERG and TOH 1953, AMIN et al. 1954). However, BONNYCASTLE et al. (1957) claim that simple mincing of the tissues with scissors before extraction with acetone allows only an incomplete extraction and that better results may be obtained if tissues are first minced with scissors and then homogenized before extraction.

On the other hand, AMIN et al. (1954) affirm that 80% acetone extracts from tissues not only 5-HT but also other substances, such as substance *P*, which are capable of interfering with the bioassay of 5-HT especially when using the rat uterus preparation. To eliminate the disturbing polypeptide they recommend extraction of the tissues with 19 parts of acetone, instead of with 4 parts, to give a final concentration of 95% acetone. This suggestion was widely accepted but it soon appeared that 95% acetone, while eliminating to a large extent the interference of substance *P*, failed to produce a satisfactory extraction of 5-HT (CORREALE 1957, BENDITT and WONG 1957).

Table 1 shows the results of unpublished experiments which have been carried out on the ox spleen and the rabbit small intestine in order to determine the suitability of the different procedures which have been suggested for the extraction of 5-HT from tissues.

The extraction procedures used were as follows:

1. the tissue was simply minced with scissors and then extracted with 4 parts of acetone (1a) or 19 parts of acetone (1b);

2. the tissue was passed through a meat grinder and then extracted with 4 parts of acetone (2a), 19 parts of acetone (2b), 4 parts of absolute ethanol (2c), 4 parts of absolute methanol (2d), or boiled for 20 min with 2 parts of distilled water and then treated with 4 parts of acetone (2e);

3. the tissue was homogenized with an equal volume of isotonic saline (homogenizer flask cooled with ice!) and the resulting homogenate extracted with either 4 parts (3a) or 19 parts of acetone (3b);

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4. the tissue was homogenized directly with 4 parts of acetone (4a) or 19 parts of acetone (4b);

5. the tissue was homogenized with 2 parts of 0.1 N HCl and, after neutralization with Na OH, the homogenate was treated with 4 parts of acetone.

Table 1. *Extraction of 5-HT from ox spleen and rabbit intestine by different solvents and extraction procedures* (explanation in the text)

Extraction procedure	Ox spleen		Rabbit intestine	
	Absolute extraction (μg 5-HT/g tissue)	Percent extraction[1]	Absolute extraction (μg 5-HT/g tissue)	Percent extraction[1]
1a	9.7	100	3.6	100
1b	3.7	38	2.1	57
2a	9.5	97	=	=
2b	4.1	42	=	=
2c	5.2	53	=	=
2d	7.3	75	=	=
2e	10.2	105	=	=
3a	9.5	97	2.6	72
3b	7.1	73	1.1	30
4a	8.1	83	3.6	100
4b	5.6	57	0.9	25
5	7.7	79	3.9	108

[1] Extraction obtained with procedure 1a is considered = 100.

The first extraction lasted 24 hr, then the material was re-extracted for another 24 hr with 3—4 parts of the same solvent, having the same final concentration.

All the extracts were assayed on the rat uterus preparation.

Recovery of 5-HT added to ox spleen homogenates, obtained with either isotonic saline or 0.1 N HCl, before addition of 4 parts of acetone, was 90—107%.

The results of some additional experiments carried out on rat tissues and on the posterior salivary glands of *Eledone moschata* are shown in Table 2.

Ox spleen and posterior salivary glands minced with scissors and then extracted twice with 95% acetone, were re-extracted with 60% acetone and then boiled with distilled water. Only a fraction of the 5-HT retained in the tissues following treatment with 95% acetone was recovered in this way.

Unlike tissue 5-HT, serum 5-HT can be extracted with 19 vol of acetone as completely as with 4 vol. Eighty per cent or, still better, 70% acetone affords a complete extraction of the indolealkylamines and phenylalkylamines contained in dry amphibian skin. It is, however, advisable to prolong extraction for 7—14 days (ERSPAMER, unpublished observations).

Table 2. *Extraction of 5-HT from rat tissues and posterior salivary glands of Eledone moschata by different procedures* (explanation in the text)

Tissue	Yield of 5-HT (μg/g) after extraction procedure					
	1a	1b	3a	3b	4a	4b
Rat brain . . .	0.7	0.27	0.55	0.25	=	=
intestine .	4.3	2.4	3.4	2.1	=	=
spleen . .	3.2	1.2	3.6	2.0	=	=
Eledone moschata posterior salivary glands .	450	140	420	180	430	160

FENSTER and TOWNE (1963) and TOWNE and KALAYIL (1964) have called attention to the possibility that lipids extracted from tissues by acetone produce a solubilization of 5-HT in lipid solvents such as petroleum ether, which is sometimes used to remove fats. This solubilization might represent a remarkable source of error in the bioassay of 5-HT.

Generally treatment of acetone extracts with petroleum ether is completely superfluous. If necessary, the eventual passage of 5-HT into ether should be checked.

The above results demonstrate that treatment with 80% acetone, preceded by mincing of the tissues with scissors, represents the most simple and convenient procedure for a complete extraction of 5-HT from the tissues of both vertebrates

and invertebrates. 95% acetone, as recommended by several investigators, can be used only for serum and possibly for other organic liquids. For tissues it is incapable of assuring a satisfactory extraction of 5-HT. In fact, only 30 to 50% of tissue 5-HT can be found in 95% acetone and the aliquot of the amine which does not pass into 95% acetone is tenaciously retained in the tissue, from which, subsequently, it cannot be completely recovered with any solvent, not even boiling water. In no instance and under no conditions do homogenized tissues give higher yields of 5-HT than tissues simply chopped up with scissors before extraction. On the contrary, especially when tissues are homogenized with saline, remarkable losses in 5-HT recovery may frequently be observed. They are probably to be ascribed to the monoamineoxidase made disposable in active form during homogenization.

Homogenization of tissues with 0.1 N HCl, as usually carried out in the spectrofluorometric estimation of 5-HT, does not produce any better extraction of the amine than 80% acetone. Besides, acid extracts cannot be stored as easily as acetone extracts.

II. Smooth muscle preparations used in the bioassay of indolealkylamines

1. Rat uterus

a) Erspamer's original method

The isolated rat uterus has been proposed by ERSPAMER for the quantitative estimation of 5-HT since 1940 (see also 1948, 1954, 1955).

Preparation of the uterus. Young rats, weighing 120—160 g, are ovariectomized and then, after a recovery period of at least a week, injected subcutaneously, on two successive days, with single doses of 100—200 μg of oestradiol dipropionate. The uterus is removed 3—5 days after the first injection and suspended in an aerated 10 ml. bath of Tyrode solution containing atropine (10^{-6}) at 29—31° C. The gimbal lever magnification is generally 4 to 6 and the tension 1 g. The composition of the Tyrode solution is as follows: sodium chloride, 8 g; potassium chloride, 0.2 g; calcium chloride, 0.2 g; magnesium chloride, 0.1 g; sodium bicarbonate, 1 g; sodium dihydrogen phosphate, 0.05 g/l. The response of the uterus preparation is recorded for 3—4 min, and doses are repeated at intervals of 6 to 7 min.

Response of the uterus. The response of the uterus usually consists of a number of contractions between which partial relaxation occurs, so that the full response consists of waves of contraction superimposed on a more or less sustained tonic contraction. Although all aspects of the uterus response must be considered during the assay, particular emphasis is laid on sustained contraction, not only on the height of this contraction but also how well it is maintained. As shown in Fig. 1 the rise in sustained contraction is satisfactorily proportional to the dose of 5-HT. Washing with fresh nutrient liquid produces immediate relaxation and prompt return to the base-line. There is no sign of tachyphylaxis or sensitization.

A good uterine preparation always responds to 5 ng 5-HT/ml and often the sensitivity increases up to 1 ng/ml (ERSPAMER 1954, SCHWARTZ 1955, LEITSCH et al. 1958). Uterine horns which do not react to 10—20 ng/ml 5-HT should be rejected. Satisfactory result are obtained in 70—80% of the experiments. According to SUPEK and MILKOVIĆ (1956) the average limit of error of the method ($P=0.05$) is 10.5%, but in the writer's opinion it may be consistently less in good preparations.

Outside the oestrus-period the uterus is considerably less sensitive to 5-HT and unsuitable for the bioassay of the amine (ERSPAMER 1954, ROBSON et al. 1954, BUSCH 1956).

Specificity of the method. The stimulant action of *acetylcholine* may be completely blocked by atropine (10^{-6}—10^{-7}). *Histamine* inhibits the rat uterus, but this inhibitory effect seems to be of no practical importance, since as much as 10 μg histamine is required to reduce by 20% the stimulant action of 0.04 μg 5-HT.

Tyramine, octopamine, dopamine, noradrenaline and *adrenaline* all inhibit the uterus preparation but practically only adrenaline deserves consideration. In fact, 0.1—0.2 μg adrenaline is sufficient to halve the response to 0.2 μg 5-HT Noradrenaline, which is certainly the catecholamine most likely to occur in tissue

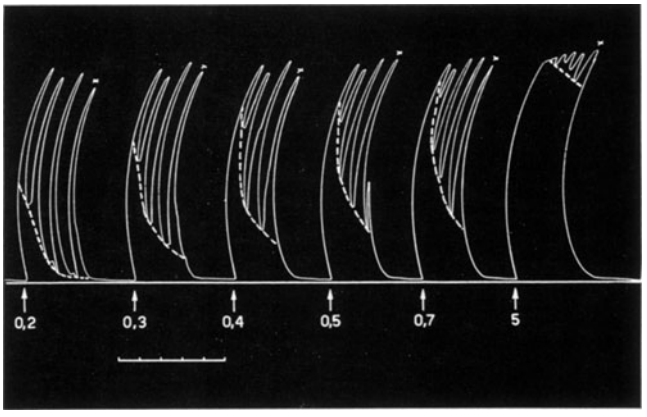

Fig. 1. The response of the rat oestrus-uterus to doses of 5-HT picrate in μg. Bioassay is based not so much on the amplitude of single contractions as on the maintenance of diastolic tone (see area between broken line and base line). A 10 ml bath containing Tyrode solution was used. Time in min. x wash. [From Fig. 23, ERSPAMER, V., Rend. sci. Farmitalia **1**, 122 (1954).]

extracts together with 5-HT, displays an inhibitory effect which is 50—100 times less intense. If catecholamines, especially adrenaline, are suspected of being present in an extract, they can be differentially destroyed by a procedure such as that used by GARVEN (1956).

Adenosine and *adenosine triphosphate* possess a stimulant action which is at least 2000—5000 times less than that of 5-HT.

Polypeptides probably represent the most important potential source of error in the bioassay of 5-HT by the rat uterus method. However, whereas the uterus is extremely sensitive to some polypeptides, it is rather insensitive to others.

Among the biogenic polypeptides which potently stimulate the uterus are angiotensin, oxytocin and the kinins, the respective threshold concentrations being approximately 1—2 ng, 0.01—0.05 milliunits, and 0.01—0.1 ng/ml (synthetic bradykinin).

A relevant example of the heavy interference of bradykinin-like polypeptides in the bioassay of 5-HT and related indolealkylamines is given by some extracts of amphibian skin which may contain amounts of kinins equiactive to 500—10.000 μg 5-HT per g tissue (ERSPAMER and co-workers, unpublished observations).

The current opinion that substance *P* may consistently disturb the assay of 5-HT in brain or intestinal extracts seems to be hardly tenable after the recent demonstration that pure substance *P* has a very poor stimulant action on the rat uterus (HAEFELI and HÜRLIMANN 1962, CLEUGH and GADDUM 1963).

Eledoisin, physalaemin and other polypeptides behave like substance *P* (ERSPAMER and FALCONIERI ERSPAMER 1962, ERSPAMER and co-workers, unpublished observations).

Fortunately, incubation with chymotrypsin at pH 7.5—8 offers the possibility of destroying the activity of all disturbing polypeptides leaving unaffected that of 5-HT and related indolealkylamines.

As research progresses the number of active compounds which are found in tissues and which are capable of interfering with the bioassay of 5-HT by the rat uterus method is increasing. It is therefore advisable to check the reliability and specificity of the method not only in the case of extracts of different biological materials but even in the case of extracts of the same material obtained from different species.

Obviously, this statement is valid not only for the rat uterus but also for all other smooth muscle preparations used in the bioassay of indolealkylamines.

b) Gaddum's technique

According to GADDUM and HAMEED (1954) and to AMIN et al. (1954) ovariectomy does not appear to be essential. Stilbestrol (0.1 mg/kg in arachis oil) is injected subcutaneously into a virgin rat weighing 160—200 g and the next day the uterus is removed and the middle 2 cm of one horn is suspended in an aerated 2 ml bath of JALON'S solution containing atropine (10^{-6}) at 30°. The composition of the JALON'S solution is as follows: sodium chloride, 9; potassium chloride, 0.4; calcium chloride, 0.06; sodium bicarbonate, 0.15; glucose, 1 g/l.

The lever magnification is about 10 and the tension 1 g weight. The time interval between doses is usually 4 min and the drug is left in the bath for 40 to 60 sec, the contact being interrupted as soon as the first contraction of the muscle has attained its maximum.

It clearly appears that the criteria adopted by the writer and by GADDUM in evaluating the uterine response are somewhat divergent.

If a higher sensitivity is required from the rat uterus then it may well be worth while trying the technique of superfusion described by GADDUM (1953).

In order to eliminate in the bioassay of 5-HT the interference of disturbing substances, SUPEK and RANDIĆ (1961) suggest enriching tissue extracts, especially brain extracts, with a known amount of exogenous 5-HT (e.g. 2 μg/g fresh tissue). By adding 5-HT artificially, the enriched extract can then be diluted to such an extent that all interfering substances are present in biologically inactive concentrations. According to SUPEK and RANDIĆ the limit of error ($P=0.05$) of assays of this type is relatively high (20%).

Instead of JALON'S solution, PUT and HOGENHUIS (1962) recommend a nutrient liquid of the following composition: sodium chloride, 8.1; potassium chloride, 0.246; calcium chloride, 0.139; magnesium chloride $+6\,H_2O$, 0.243; sodium bicarbonate, 1.76; sodium dihydrogen phosphate $+\,H_2O$, 0.066; urea, 0.132; glucose, 0.611; and atropine sulphate, 0.001 g/l. Bath temperature 30°.

2. Rat fundus strip

Preparation of the fundus strip. The stomach is dissected free from the abdomen and dropped into the bathing solution. The pyloric antrum is removed, except for a small band, and the fundal content washed away. The fundus, which appears as a translucent balloon-like tissue, is then cut into a strip by opening the fundus along the lesser curvature and cutting to preserve the longitudinal musculature. The resultant strip is then hung in a small bath and the movements

recorded by an isotonic lever. It is convenient to leave a small band of pyloric tissue still attached to the fundus, to act as a marker, and so prevent cutting in the wrong direction after the fundus has been opened into a flat plate (Fig. 2).

After the strip has been suspended it is important for it to be allowed to recover and relax for 15 to 60 min before starting stimulation. During this period the bathing solution is either allowed to perfuse through the organ bath at a rate of about 1 ml/min or is changed by overflow at about 6-min intervals.

Bath and bathing solutions. Generally a bath 15 cm long holding 5 to 10 ml of fluid is used. Of the various bathing solutions which have been tried the most

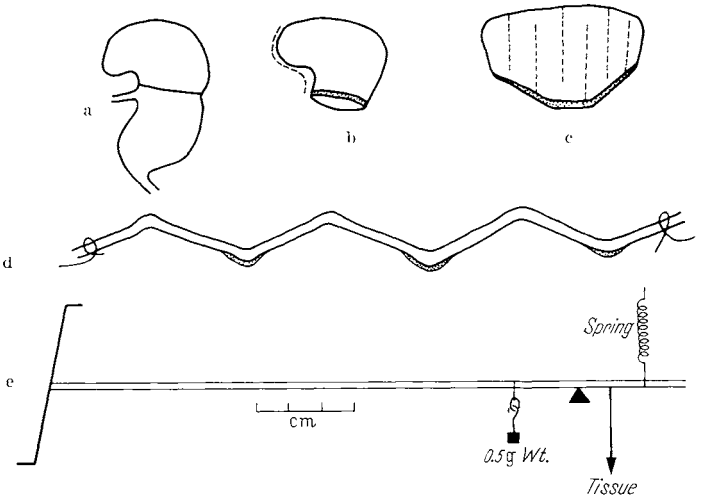

Fig. 2a—e. Diagram showing the method of cutting the rat fundal strip and the arrangement of the lever. (a) The whole stomach. (b) The fundus, left with a thin band of pyloric tissue and cut along the dotted line. (c) The fundus opened into a plate of tissue and cut along the dotted lines. (d) The strip pulled out by cotton tied to each end, so that protrusions of bits of pyloric tissue can be cut off. (e) The lever, showing the relative positions of the load, the tissue and the spring. Without any of these, the lever should come to rest horizontally. The scale refers to the lever only. [From Fig. 1, VANE, J. R., Brit. J. Pharmacol. **12**, 345 (1957).]

suitable seem to be the two following, recommended by VANE (1957) and UUSPÄÄ and UUSPÄÄ (1962), respectively:

a) rat uterus Ringer: sodium chloride, 9; potassium chloride, 0.4; calcium chloride, 0.06; sodium bicarbonate, 0.15; glucose, 1 g/l.

b) sodium chloride, 8; potassium chloride, 0.4; calcium chloride, 0.17; sodium bicarbonate, 0.3; glucose, 0.3 g/l.

In both Krebs and Tyrode solutions the muscle is slightly more sensitive to 5-HT, but the spontaneous movements are greater than in rat uterus Ringer solution.

The bathing solution is actively aerated with oxygen at 37°. Lower temperatures make the muscle less sensitive, while higher temperatures elicit greater spontaneous movements. Washing of the preparation is always by overflow.

Lever. A balsa wood lever with a frontal writing point, completed with a light spring and a vibrator was originally used by VANE (1957), but later on abandoned in favour of a simple Palmer lever with an all-metal frontal writing point and PATON's pendulum. Magnification is about 15-fold and the load on the preparation varies between 1 and 3 g (VANE 1958, UUSPÄA and UUSPÄÄ 1962).

Assay cycle. 5-HT is added to the bath and the contraction is allowed to develop for 90—120 sec. The bath is then washed out and a weight added to the lever so that it comes to rest immediately on a bar fixed at a predetermined level.

After a constant time (15 to 30 sec depending on the individual muscles) the extra weight is removed. The tissue rapidly regains its basal length and after a further 2 min the next dose is added. Stretching the muscle to a fixed length is necessary owing to excessive slowness of spontaneous relaxation.

It also seems possible, when the writing point does not fall to the base line spontaneously, to drop the lever by gently knocking against the working table (UUSPÄÄ and UUSPÄÄ 1962).

The greatest difficulties in using the fundus strip arise from the already mentioned slowness of relaxation after a dose of 5-HT and from changes in the resting length of the muscle. However, when very high sensitivity is not needed and, hence, it is not required to use only the bottom part of the dose/response

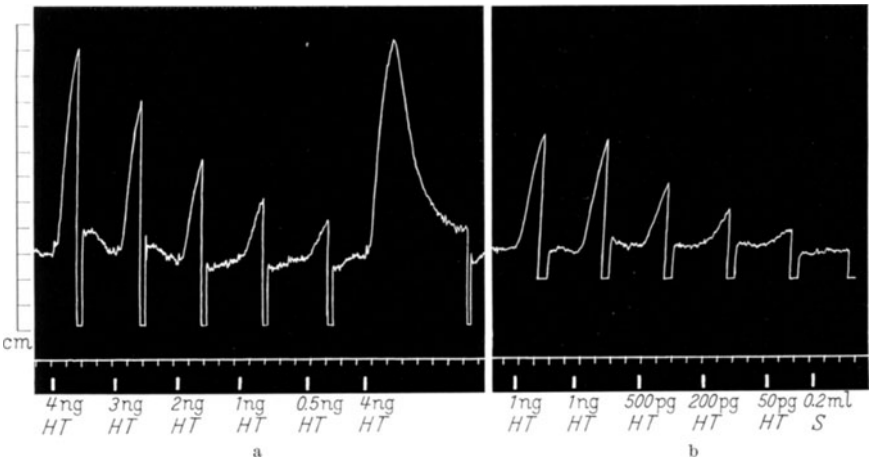

Fig. 3a and b. (a) The response of the rat fundal strip to doses of 5-HT in nanograms (ng). The drum was left running continuously to illustrate the cycle. The second dose of 4 ng 5-HT shows the slowness of relaxation if the tissue is not stretched. (b) A more sensitive preparation showing responses down to 50 picagrams (pg) 5-HT, with an injection of 0.2 ml saline (*S*) at the end. A 5 ml bath containing rat uterus Ringer at 37° was used. The vertical scale is in cm. Time in min. [From Fig. 2, VANE, J. R., Brit. J. Pharmacol. **12**, 346 (1957).]

curve, then it is not necessary to maintain a stable baseline during the assay and, consequently, the preparation becomes easier to handle.

Sensitivity to 5-HT. The most sensitive preparations may give a measurable response even to 0.01 ng 5-HT/ml; tissues with average sensitivity respond to 0.02—0.06 ng 5-HT/ml (VANE 1957) (Fig. 3).

Specificity of the method. According to VANE the following doses of other biogenic compounds are required to produce a contraction similar to that given by 1 ng 5-HT: acetylcholine, 10 ng; histamine, more than 1 μg; adenosine triphosphate, 20 μg; pitressin, 0.4 units; potassium chloride, 2 mg; tryptamine hydrochloride 0.25 μg; 4-hydroxytryptamine 1.8 ng; 5-methoxytryptamine, 20 ng. In their turn ERSPAMER and FALCONIERI ERSPAMER (1962) found that 1 ng 5-HT was equivalent to approximately 25—50 ng eledoisin, 250—1000 ng bradykinin and 3—6 units substance *P*.

Sympathomimetic amines relax the fundal strip and reduce the response to 5-HT. For example, 25—30 ng of either adrenaline or noradrenaline are sufficient to halve the effect of 5 ng 5-HT. In order to render the muscle insensitive to acetylcholine, hyoscine hydrobromide or atropine sulphate (10^{-7}) is added to the bathing fluid. The interference of polypeptides and catecholamines may be excluded in the manner previously described.

Assay precision. According to UUSPÄÄ and UUSPÄÄ the limit of error of the method corresponding to 5% level of significance is 8.8%.

Advantages and disadvantages of the fundus strip in comparison with the uterus. The main advantages of the fundus strip over the rat uterus are, according to VANE (1957), that it does not need pretreatment of the donor rat, that it will work in several bathing solutions, and that it can always be relied upon to respond to 0.2 ng and very often to 0.02 ng 5-HT per ml bathing fluid.

Actually, the only important advantage is that of the greater sensitivity of the fundus strip. This preparation is decidedly to be preferred whenever 5-HT is present in low concentrations and the interference of disturbing substances may be excluded. The other advantages are either inconsistent or largely neutralized by the tedious cutting of the fundal strip, and the difficulty of relaxation of the preparation, requiring stretching and removing of extra weights from the lever. It should be added that the choice between stomach fundus and uterus is not only a matter of sensitivity, but also of specificity. The rat stomach, for example, is relaxed by noradrenaline and contracted by substance *P*, eledoisin and eledoisin-like polypeptides much more potently than the rat uterus and this, conversely, is more sensitive to bradykinin-like polypeptides and to oxytocin than the fundus strip.

3. Rat colon

This preparation was first recommended for the assay of 5-HT by DALGLIESH et al. (1953).

The part of the rat colon nearest the caecum is suspended in 5—20 ml of a Ringer solution of the following composition: sodium chloride, 9; potassium chloride, 0.4; calcium chloride, 0.03; sodium bicarbonate, 0.15; and glucose, 1 g/l. The temperature of the bath is 22—24°. Test substances are added to the bath at 4—5 min intervals and kept in the bath for 1.25—1.5 min.

According to SUPEK and MILKOVIĆ (1956) the average concentration of 5-HT producing a suitable response by the colon preparation is 20—30 ng/ml. It may be seen that this concentration is higher than that required to elicit a similar response by the rat uterus.

The atropinized rat colon is practically insensitive not only to acetylcholine but also to histamine, while giving moderate responses to the usual biogenic polypeptides.

SUPEK and MILKOVIĆ found that the average limit of error ($P = 0.05$) of this method is 8.8%, and they affirm that the colon is to be preferred to the uterus in the assay of 5-HT. This opinion, however, is not shared by GADDUM and HAMEED (1954) nor by the writer.

4. Guinea-pig ileum

Preparation of the ileum. The lowest piece of the gut next the colon is found especially sensitive and animals which have fasted for 18 hr provide the best preparations. The gut is suspended in a 2—10 ml bath containing Tyrode or Krebs solution maintained at 30—37° and the movements of the longitudinal muscle are recorded with a common gimbal lever or with a lever with a frontal writing point. During assays the drug is left in the bath for 40 sec and doses are repeated at intervals of 4—5 min (AMIN et al. 1954, GADDUM and HAMEED 1954, BARLOW and KAHN 1959).

Sensitivity and specificity of response. Not only is the sensitivity of this preparation at least 10 times less than that of the rat uterus but, what is more important, the specificity of the response is unsatisfactory. It is difficult to find

another smooth muscle preparation which is contracted by such a variety of active tissue constituents as the guinea-pig ileum is. To remain in the field of polypeptides, the preparation is stimulated by vasopressin, angiotensin, bradykinin-like polypeptides, substance *P*, eledoisin, physalaemin etc. In addition the guinea-pig ileum may be extremely sensitive to lipid-soluble organic acids such as prostaglandin, vesiglandin, darmstoff, irin, slow-reacting substance *A* etc., with the disadvantage that these compounds cannot be inactivated by proteolytic enzymes.

On the whole it may be stated that the guinea-pig ileum is unsuitable not only for the quantitative estimation of 5-HT but even for the qualitative detection of the amine in crude biological materials.

5. Isolated rabbit ear

The isolated rabbit ear is perfused at room temperature through a cannula in the central artery. A special injection device is interposed between the reservoir

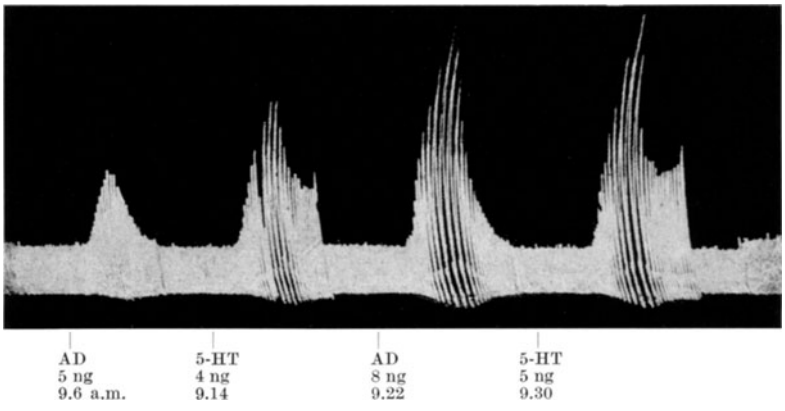

Fig. 4. The response of the perfused rabbit's ear to doses of 5-HT and adrenaline (AD) in nanograms (ng). Height of record shows interval between drops. It may be noted that the vasoconstrictor effect of 5-HT is more potent than that of adrenaline. [From Fig. 5, GADDUM, J. H., and K. A. HAMEED, Brit. J. Pharmacol. **9**, 244 (1954).]

containing the perfusion fluid and the arterial cannula. The ear is placed on a draining plate and the perfusate from the cut surface is led to a drop counter system.

The most highly recommended perfusion fluid is that used by PAGE and GREEN (1948) for the study of serum vasoconstrictor. It has the following composition: sodium chloride, 8.2; potassium chloride, 0.84; calcium chloride + 2 H_2O, 0.04; magnesium chloride + 6 H_2O, 0.06; sodium bicarbonate, 0.4; and glucose, 1 g/l. To each litre is added 10 ml of phosphate buffer containing 4 parts of 1 *M* K_2HPO_4 to 1 part of 1 *M* KH_2PO_4.

When this solution is used the ear is much more sensitive to vasoconstrictor substances than when Locke's solution is used. The ear is always more sensitive the day after being kept overnight in the refrigerator.

The sensitivity of the preparation to 5-HT is of the order of 1 ng or even less (GADDUM and HAMEED 1954, BERTACCINI and ZAMBONI 1961) (Fig. 4).

However, the rabbit ear, while presenting possibilities of advantageous use in the assessment of the relative potencies of pure 5-HT-like substances and in the study of anti-5-HT drugs, does not seem to be suitable for the quantitative estimation of 5-HT or other indolealkylamines in crude tissue extracts.

The preparation is, in fact, tremendously sensitive to catecholamines and remarkably sensitive to other biogenic amines as well as to vasoactive polypeptides and, as a consequence, its response is by no means specific for 5-HT.

6. Molluscan heart

a) Venus mercenaria

The isolated heart of *Venus mercenaria* was first recommended by WELSH and TAUB in 1955, and since then it has become the molluscan heart most frequently used in the bioassay of 5-HT.

Method for preparing the heart. Specimens of *Venus mercenaria* with a shell length of 8 to 12 cm have been found to be the most convenient size. After removal from the sea they may be stored for one or two weeks in shallow tanks of aerated sea water, at 15—18°, or kept dry at 5—10°.

The heart is exposed by breaking and removing the dorsal portion of the shells (umbos and hinge) and then cutting away the mantle and pericardium dorsal to the heart. The heart consists of a single median ventricle with laterally disposed, thin-walled atria. Anterior and posterior blood vessels leave the heart in close association with the intestine which passes through the heart. Threads for attaching it to a support in the bath and to a writing lever may be passed under the atria and tied close to the ventricle in order to include some of the thicker-walled ventricular muscle. Cutting the atria distal to the threads and cutting the bloods vessels and the intestine isolate the ventricle, which may then be placed in an appropriate heart bath. A bath holding 10 ml when filled has been found appropriate. The heart lever should be counterweighted to give a pull of 200 to 300 mg.

According to MCINTYRE et al. (1960), for best results the heart should be suspended so that the contiguous segment of gut lies transversely and not parallel with the contraction axis of the heart.

Sea water thermostatically maintained at 15—18° is an adequate perfusion fluid for the isolated heart. Another fluid found satisfactory has the following composition: sodium chloride, 30; potassium chloride, 0.9; calcium chloride, 1.1; magnesium sulphate $+3\,H_2O$, 3.5 g/l; with a phosphate or bicarbonate buffer (pH 7—7.5). Oxygen may be supplied by air or a mixture of 95% O_2 and 5% CO_2 passed through the bath (WELSH and TAUB 1955).

Response of the heart. The response of the *Venus* heart to 5-HT has three components: an increase in amplitude, an increase in frequency, and an increase in the resting tone of the muscle. The relative importance of these components varies with the concentration. Between threshold concentration (about 10^{-10}) and moderately high doses (about 10^{-7}) the response of the *Venus* heart to 5-HT is mainly an increase in the amplitude of beat. There is also an increase in frequency which, however, does not seem to be dependable. Above 10^{-7} 5-HT the relative increase of the tone becomes larger, and at 10^{-6} the effect is almost all increase in tone while rhythmical excursions of the heart are very small (Fig. 5).

The bioassay of 5-HT is mainly based on the difference between the amplitude of beat before and after the addition of the drug. Thus, the most reliable results are obtained with concentrations of 5-HT varying between 1 and 10—20 ng/ml. With these concentrations the heart amplitude varies linearly with the log concentration (WELSH and TAUB 1955, WELSH 1957, GREENBERG 1960a, 1960b, MCINTIRE et al. 1960).

Specificity. There are, on the whole, meagre data on the specificity of the response of the *Venus* heart to 5-HT. However, it should be emphasized that:

(a) acetylcholine displays on the *Venus* heart a powerful inhibitory action, the threshold concentration being 10^{-10} to 10^{-11}. If present, the disturbing action of acetylcholine may be eliminated either by benzoquinonium chloride (10 μg/ml) or by enzymatic hydrolysis.

(b) the *Venus* heart is 5- to 30-fold more sensitive to bufotenine and twice as sensitive to N-methyl-5-HT as to 5-HT itself (Bumpus and Page 1955, Greenberg 1960b). Thus, bioassay of 5-HT is impossible in the presence of these N-methylated derivatives. This statement is valid also when the *Helix* heart is used instead of the *Venus* heart (Bertaccini and Zamboni 1961).

(c) both invertebrate and vertebrate tissues and fluids may contain unknown substances capable of stimulating the *Venus* heart or of increasing its sensitivity to 5-HT and hence of invalidating any attempt at quantitative estimation of the amine (Welsh 1956, Bowers 1962).

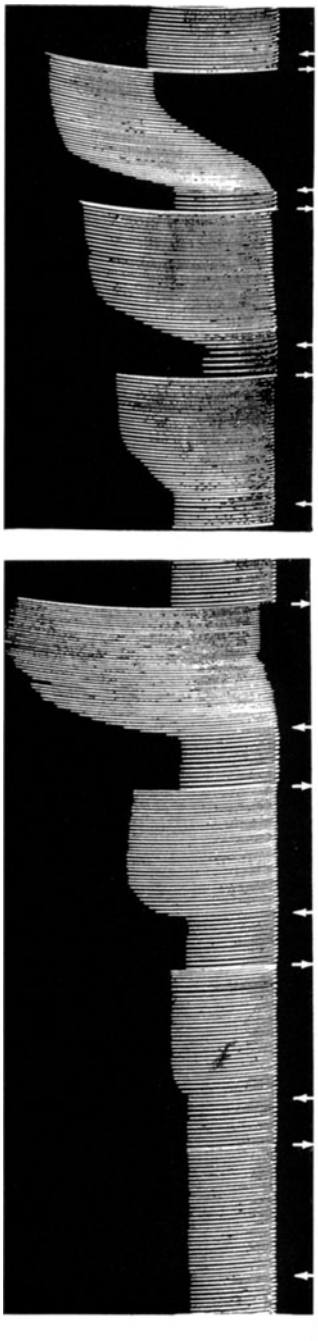

Fig. 5. The action of different concentrations of 5-HT on two preparations of *Venus nereonaria* heart. At each ↓, drum was stopped and bath flushed with sea-water for 5 min. Note the effect of increasing concentrations of 5-HT: at first increase in amplitude and frequency of beat may be seen, then increase in diastolic tone. [From Figs. 3 and 6, Wright, A. M. et al., Brit. J. Pharmacol. **18**, 444, 448 (1962).]

b) Helix (several species)

The ventricles of *Helix aspersa*, *Helix pomatia* and *Helix luconum* are tied by ligatures in the ventriculo-aortic and auriculo-ventricular grooves to the light lever and a fixed support; a small piece of the auricle is retained because this seems to produce a more regular beat. The heart is suspended at room temperature in a 2—3 ml bath, bubbled through with air.

Several perfusion fluids have been recommended. The composition of three of them is as follows:

α) sodium chloride, 6.5; potassium chloride, 0.2; calcium chloride, 0.2; magnesium chloride, 0.1; sodium dihydrogen phosphate, 0.05; sodium bicarbonate 1 g/l (Zetler and Schlosser 1954).

β) sodium chloride, 5; potassium chloride, 0.2; calcium chloride, 0.2; magnesium sulphate $+7\ H_2O$, 0.3; sodium dihydrogen phosphate, 0.1; sodium bicarbonate, 0.1 g/l (Bertaccini and Zamboni 1961).

γ) sodium chloride, 3.45; potassium chloride, 0.43; calcium chloride, 1.17; magnesium chloride $6\ H_2O$, 3.31; sodium bicarbonate 1.1 g/l (Kerkut and Cottrell 1963).

Change of the solution is by overflow.

The threshold concentration of 5-HT causing increase in amplitude and frequency of beat is normally about 1 ng/ml but at times the snail heart can be sensitive even to 0.01 ng/ml 5-HT (KERKUT and COTTRELL 1963). The maximum reaction is reached in about 20 sec and graded responses may be obtained.

Adrenaline and histamine are without effect, but acetylcholine inhibits the heart in about the same doses as 5-HT stimulates it. This effect is not blocked either by atropine or benzoquinonium (10 μg/ml) (GADDUM and PAASONEN 1955, ERSPAMER and GHIRETTI 1951, BERTACCINI and ZAMBONI 1961).

c) Spisula (Mactra) solida

The heart of this bivalve mollusc has been especially recommended in the bioassay of 5-HT by GADDUM and PAASONEN (1955).

The *Spisula* specimens are opened by cutting both adductor muscles with a thin scalpel, and then dissecting by the technique of WELSH and TAUB (1948). Threads are tied near the auriculo-ventricular junctions and a small piece of ventricle is included in the ligature because of the fragility of the auricles.

The heart is then suspended at room temperature (13—21°) in a 2 ml bath, the contents of which can be changed by overflow so that the mechanical disturbance is small. Air is always bubbled through the fluid and the movements of the heart are recorded on a smoked drum with a light lever.

The nutrient solution has the following composition: sodium chloride, 23; sodium sulphate, 4; potassium chloride, 0.65; calcium chloride, 1.1; magnesium chloride · 6 H_2O, 10; sodium carbonate, 0.2 g/l. Sometimes the heart gives a better beat when the concentration of Mg is reduced by 2—5 times.

The heart of *Spisula* starts to beat in 5—60 min after dissection at a rate of 12—25 beats per minute. 5-HT increases the amplitude of beat in concentrations as low as 0.1—0.5 ng/ml. Maximal response is reached within 45 to 60 sec and relaxation is rapid. Graded responses are obtained within large dose limits and for the maximum effect the concentration of 5-HT is 1 μg/ml.

Acetylcholine usually inhibits the *Spisula* heart in threshold concentrations of 1—100 ng/ml, the only efficient antagonist of this action being benzoquinonium (1—10 μg/ml). Adrenaline and noradrenaline stimulate the heart in concentrations of 1 μg/ml or more. Histamine is ineffective at this dose level. Adenosine, adenosine-5-phosphate and adenosine diphosphate display a very small stimulant action only in concentrations of 1 mg/ml. Crude preparations of substance *P* and bradykinin are inactive.

d) Anodonta cygnea

The isolated ventricle containing a short piece of intestine is suspended in a 10 ml bath bubbled through with air. The nutrient liquid consists of a mixture of 96 parts of distilled water and 4 parts of sea water buffered by *M*/400 sodium bicarbonate.

The *Anodonta* ventricle, either immediately or after an initial standstill of varying duration, begins to beat with a slow even rhythm. Generally the isolated ventricles are able to continue beating for more than 48 h.

The threshold concentration of 5-HT causing increase in amplitude and frequency of beat is about 1 ng/ml, and graded responses are obtained with concentrations up to about 1 μg/ml.

Adrenaline, noradrenaline, tyramine, dopamine, substance *P*, histamine and acetylcholine are either ineffective or produce negligible responses at high concentrations (FÄNGE 1955, MARCZYNSKY 1959).

e) Other molluscs

Hearts of other bivalve molluscs which may be used in the bioassay of 5-HT are those of *Cyprina islandica* (threshold concentration of 5-HT 1 ng/ml), *Mya arenaria* (0.5—50 ng/ml) and *Cardium edule* (0.1—1 ng/ml) but, on the whole, for one reason or another, they do not seem to be as convenient as hearts of *Spisula* or *Venus*. The same dissection technique as described for the *Spisula* heart in used.

No satisfactorily beating hearts have been obtained from *Barnea candida*, *Mactra corallina*, *Mytilus edulis* or *Paphia saxatilis* (GADDUM and PAASONEN 1955).

Hearts of *Buccinum undatum* and of *Octopus* or *Eledone* are employed more rarely in the bioassay of 5-HT. The heart of *Buccinum* is cannulated with a Straub cannula through the aorta and the auricle is tied off (GADDUM and PAASONEN 1955); the heart of *Octopus* is cannulated with a similar cannula which is introduced through one of the atria, all the vessels which open into the ventricle having been ligatured first (ERSPAMER and GHIRETTI 1951).

7. Miscellaneous preparations

Guinea-pig uterus. According to MCGOVERN et al. (1961) uterine muscles from guinea-pigs in natural oestrus may serve excellently for the qualitative and quantitative bioassay of 5-HT. 0.5 μg/ml 5-HT gives maximal response.

Perfused pig ear. 5-HT, adrenaline and noradrenaline, in doses from 0.01 to 0.1 μg. produce vasoconstriction proportional to the dose and suitable for recording (GRETTE 1957).

Carotid artery rings or strips. They have been widely used in the past, and more recently by WOOLLEY and SHAW (1953), but have now been completely abandoned owing to the difficulty of obtaining repeatable results, and their poor sensitivity and specificity.

Anterior retractor muscle of the byssus. The superfused isolated anterior retractor muscle of the byssus of *Mytilus edulis* may be used for the identification of 5-HT owing to the capacity of the amine (1—100 ng/ml) to provoke a relaxation of the contraction produced either by electrical stimulation or by acetylcholine (CAMBRIDGE and HOLGATE 1955).

Dorsal muscle of the leech. It has been found by POLONI (1951) that 5-HT causes relaxation of the isolated leech muscle and that this effect can be utilized for its assay in concentrations as low as 0.01 ng/ml. The relaxing effect of 5-HT on the leech muscle has been confirmed by SCHAIN (1961), but it has not been found suitable as a basis for the assay or identification of the amine, mainly because of the difficulty in establishing a stable base line for comparison of relaxations and because of the lack of specificity of the response.

Isolated stomach of Helix pomatia. The isolated stomach of *Helix pomatia* is potently stimulated by 5-HT at exceptionally low concentrations (threshold 5×10^{-8} to 1×10^{-7} μg/ml) and the response is fairly proportional to the dose of 5-HT. Acetylcholine, adrenaline, noradrenaline, histamine, vasopressin and oxytocin all cause similar contraction of the snail stomach but their action is at least 10,000 times less intense than that of 5-HT. In view of these facts, grounds exist, according to GRYGLEWSKI and SUPNIEWSKI (1963) for undertaking the elaboration of a method for bioassay of 5-HT in body fluids and tissues using the above preparation.

III. The relative potency of natural and synthetic indolealkylamines

As a direct consequence of the enormous interest raised by 5-HT and the other naturally occurring indolealkylamines, numerous related indole derivatives have been synthesized and studied.

Detailed results of these studies will be reported in due course in chapter 7. Here we wish only to summarize in Tables 3 and 4 the relative activities of the most important indolealkylamines and indazolealkylamines, as assessed on some of the smooth muscle preparations most commonly used in the bioassay of 5-HT.

The following indole aminoacids and acids have proved to be inactive or at least 10,000 times less active than 5-HT on the smooth muscle preparations employed: L-tryptophan, 5-HTP, 4-HTP, 6-HTP, 7-HTP, 2-HTP, 5-hydroxy-N-acetyl-TP, 5-acetoxy-N-acetyl-TP, 5-hydroxy-N,N-dimethyl-TP, 5-hydroxy-2-methyl-TP, 5-amino-TP, 5-nitro-TP, IAA, 5-HIAA, 4-HIAA, 7-HIAA, 6-HIAA and 5-methoxy-IAA.

It may easily be seen from the tabulated data that no general conclusion is possible in regard to the relationship between chemical constitution and pharmacological activity. Therefore discussion will be limited to some of the data collected on the rat uterus and rat stomach preparations, which behave in a fairly similar manner:

a) Shifting of the phenolic-OH group from position 5 to other positions regularly produces a decay of biological activity, which is moderate when the transposition is to position 4, but tremendous when the transposition is to position 6 or 7.

b) Substitution on the indole nucleus of groups other than —OH groups regularly reduces biological activity. The reduction is moderate in the case of amino- and methoxy-groups, but considerable in the case of halo-groups and benzyloxy-groups.

c) Etherification of the phenolic —OH with glucuronic or sulphuric acid practically abolishes biological activity.

d) Lack of any phenolic —OH group, as in tryptamine, is accompanied by a strong reduction in biological activity which, however, is much less than that observed following transposition of the —OH group from position 5 to position 6 or 7 of the indole nucleus.

e) Shifting of the side chain from position 3 to position 2 produces a complete loss of biological activity.

f) The effect of alkyl substitution on the amino nitrogen is variable; in general both an increase in number and size of the alkyl group produces a reduction of biological activity.

g) Substitution of a methyl group on the α-carbon of the side chain, i.e. introduction of a third carbon atom into the side chain, produces either a moderate decay of activity or an increase in activity, depending upon other substituents in the molecule. Substitution of two methyl groups or of one ethyl group is considerably more deleterious.

h) In sharp contrast to what usually happens with phenylalkylamines, substitution of an —OH group on the β-carbon of the side chain produces the practical disappearance of any activity on the rat uterus preparation.

i) Indolealkylamines with a single carbon atom side chain (gramine derivatives) show negligible activity on all preparations tested.

k) Indazolealkylamines show remarkable 5-HT-like activity, which is particularly evident with the first member of the series, more strictly related to 5-HT from a chemical point of view.

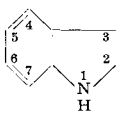

Table 3. *Equipotent molar ratios of indolealkylamines estimated on five isolated smooth muscle preparations.* 5-HT taken as unity.

Ring substituent	Side chain	Stimulant action				
		Rat uterus[1,2]	Rat stomach[1,3]	Molluscan heart[2,4]	Rabbit ear[2]	Guinea-pig ileum[2]
5—OH	3—CH_2—CH_2—NH_2 (5-Hydroxytryptamine)	1	1	1	1	1
5—OH	3—CH_2—CH_2—$NHCH_3$	1	6.3	=	1.4	7
5—OH	3—CH_2—CH_2—$N(CH_3)_2$ (Bufotenine)	4	10	0.3 (0.028)	11	4
5—OH	3—CH_2—CH_2—$N(C_2H_5)_2$	9	18; 20	=	=	=
5—OH	3—CH_2—CH_2—$N(C_3H_7$ iso$)_2$	16	4.5	=	=	=
5—OH	3—CH_2—CH_2—$N(C_3H_7)_2$	39	16; 4	=	=	=
5—OH	3—CH_2—CH_2—$N(C_4H_9)_2$	540; 1100	151	0	0	450
5—OH	3—CH_2—CH_2—$N(CH_3)_3$ (Bufotenidine)	>1000[5]	=	=	=	=
5—OH	3—CH_2—$CH(CH_3)$—NH_2	1.3; 2	1.4; 1.7	8.2 (6)	3.3	8
5—OH	3—CH_2—$C(CH_3)_2$—NH_2	310	=	170	0	24
5—OH	3—CH_2—$CH(C_2H_5)$—NH_2	50	7	50	40	44
5—OH	3—CH_2—CH_2—$NHCOCH_3$	+—[6]	=	=	=	=
5—OH	3—$CHOH$—CH_2—$N(CH_3)_2$	0	=	0	0	0
5—OH	3—CH_2—$C(N)$—NH_2	0	=	=	108	=
5—OH, 2,3-dihydro	3—CH_2—CH_2—NH_2	10[7]	=	=	=	=
5—OH	2—CH_2—CH_2—NH_2	0	=	0	0	190
5—OCH_3	3—CH_2—CH_2—NH_2 (5-Methoxytryptamine)	9.6; 8	20; 39	0.5	5.4	9
5—OCH_3	3—CH_2—CH_2—$NHCH_3$	150	=	=	=	=
5—OCH_3	3—CH_2—CH_2—$N(CH_3)_2$	150	=	=	=	=
5—OCH_3	3—CH_2—CH_2—$N(C_4H_9)_2$	0	=	540	0	0
5—OCH_3	3—CH_2—$CH(CH_3)$—NH_2	1.5; 4.3	2.9; 1.4	4.6	1.5	43
5—OCH_3	3—CH_2—CH_2—$NHCOCH_3$ (Melatonin)	0	=	=	345	0
5—$OCOCH_3$	3—CH_2—CH_2—NH_2	=	=	(+)[8]	=	=
5—CH_3	3—CH_2—CH_2—NH_2	173	184; 620	=	=	=
5—CH_3	3—CH_2—$CH(CH_3)$—NH_2	1300; 240	14; 22	190	340	0
5—Cl	3—CH_2—CH_2—NH_2	=	100	=	=	=
5—NH_2	3—CH_2—CH_2—NH_2	11	=	1.3	23	22
5—OC_7H_7	3—CH_2—CH_2—$N(CH_3)_2$	20000	625	=	=	=
5—OC_7H_7	3—CH_2—CH_2—$N(C_2H_5)_2$	10000	470	=	=	=
5—OC_7H_7	3—CH_2—CH_2—$N(C_3H_7)_2$	20000	614	=	=	=
5—OC_7H_7	3—CH_2—CH_2—$N(C_4H_9)_2$	20000	20000	=	=	=
5—OC_7H_7	3—CH_2—$CH(CH_3)$—NH_2	20000	63	=	=	=
5—O-sulphate	3—CH_2—CH_2—$N(CH_3)_2$ (Bufoviridine)	500	=	=	0	1000
5—O-glucuronide	3—CH_2—CH_2—NH_2	0	=	0	=	0
5—O-glucuronide	3—CH_2—CH_2—$N(CH_3)_2$	0	=	0	=	0
5—OH, 2—CH_3	3—CH_2—CH_2—NH_2	=	=	(31.4)	=	=
5—OCH_3, 2—CH_3	3—CH_2—CH_2—NH_2	=	=	(43.8)	=	=
5—OCH_3, 2—CH_3, 1—C_7H_7 (BAS)	3—CH_2—CH_2—NH_2	=	=	(30)[9]	=	=
5—OH, 2—CH_3, 1—C_7H_7 (BAS-phenol)	3—CH_2—CH_2—NH_2	=	=	(300)[9]	=	=
5,6—$(OH)_2$	3—CH_2—CH_2—NH_2	=	~1[10]	=	=	=
5,6—$(OCH_3)_2$	3—CH_2—CH_2—NH_2	58	300	4	140	300
5,6,7—$(OCH_3)_3$	3—CH_2—CH_2—NH_2	110	=	130	560	145
6—OH	3—CH_2—CH_2—NH_2	0	460	47	155	0
6—OCH_3	3—CH_2—CH_2—NH_2	0	1520	174	480	400
4—OH	3—CH_2—CH_2—NH_2	11	1.8	15	22	26

Table 3. (cont.)

Ring substituent	Side chain	Stimulant action				
		Rat uterus[1,2]	Rat stomach[1,3]	Molluscan heart[2,4]	Rabbit ear[2]	Guinea-pig ileum[2]
4—OH	3—CH_2—CH_2—$N(CH_3)_2$ (Psilocin)	10—20[11]	0[11]	=	=	=
4—O-phosphate	3—CH_2—CH_2—$N(CH_3)_2$ (Psilocybin)	330; 50—100[11]	3—1000[11]	18	0	760
7—OH	3—CH_2—CH_2—NH_2	0	=	0	1300	0
2—CH_3	3—CH_2—CH_2—$N(CH_3)_2$	20000	1200; 6600	=	=	=
2—CH_3	3—CH_2—CH_2—$N(C_2H_5)_2$	1000	280	=	=	=
2—CH_3	3—CH_2—CH_2—$N(C_3H_7)_2$	630	70	=	=	=
1—CH_3	3—CH_2—CH_2—NH_2	142	=	0	330	520
1—CH_3	3—CH_2—$CH(CH_3)$—NH_2	1350	69	=	=	=
	3—CH_2—CH_2—NH_2 (Tryptamine)	176; 210	408; 933	22 (9.9)	20	280
	3—CH_2—CH_2—$NHCH_3$	128; 2000	1120; 2400	(3.7)	17	48
	3—CH_2—CH_2—NHC_2H_5	2000	250; 350	(9.1)	=	=
	3—CH_2—CH_2—NHC_3H_7	0; 2000	330; 490	0	0	0
	3—CH_2—CH_2—$N(CH_3)_2$	200; 1000	196; 350	107 (10.7)	35	58
	3—CH_2—CH_2—$N(C_2H_5)_2$	160; 500	83; 112	650 (7.9)	0	0
	3—CH_2—CH_2—$N(C_3H_7)_2$	95; 200	34; 40	59	0	0
	3—CH_2—CH_2—$N(C_3H_7$ iso$)_2$	520	53	=	=	=
	3—CH_2—CH_2—$N(C_4H_9)_2$	720; 500	244	810	0	870
	3—CH_2—$CH(CH_3)$—NH_2	0; 800	31; 60	0 (8.6)	0	0
	3—CH_2—$CH(C_2H_5)$—NH_2	20000	4600	=	=	=
	3—CH_2—$C(CH_3)_2$—NH_2	=	1400	=	=	=
	3—CH_2—CH_2—$NHCOCH_3$	0	=	=	=	=
	3—$CHOH$—CH_2—$NHCH_3$	0	=	23	370	0
	3—CH_2—$C(N)$—NH_2	0	=	=	120	=
	3—CH_2—CH_2—CH_2—NH_2	=	1920	=	=	=
	3—CH_2—CH_2—CH_2—$N(CH_3)_2$	=	=	(2000)	=	=
	3—CH_2—NH_2	=	29000	=	=	=
	3—CH_2—$N(CH_3)_2$ (Gramine)	0	=	0 (0)	0	400

Values in parentheses have been obtained on the heart of *Venus mercenaria* (GREENBERG 1960b), the others on the heart of *Helix luconum* (BERTACCINI and ZAMBONI 1961).

References to Table 3. [1] BARLOW and KAHN (1959a, 1959b). — [2] BERTACCINI and ZAMBONI (1961). — [3] VANE (1959). — [4] GREENBERG (1960b). — [5] ERSPAMER (1954). — [6] MCISAAC and PAGE (1958). — [7] FELLMAN et al. (1962). — [8] OSBORNE et al. (1962). — [9] WOOLLEY (1959). [10] COLHOUN (1963). — [11] WOOLLEY and CAMPBELL (1962).

The aldehydes corresponding to 5-HT and tryptamine, generated *in vitro* by purified oxidative deaminase preparations and isolated free of amines, do not show any activity on the rat uterus and the guinea-pig ileum at concentrations several hundred times higher than the parent amines nor do they possess any inhibitory or potentiating effect on the activity of the amines. Since the aldehydes are taken up and metabolized by the smooth muscle preparations, their inactivity is not due to impermeability (RENSON et al. 1964).

VANE (1959) has observed that the relative potency of many of the examined indolealkylamines changes considerably following introduction of a MAO inhibitor into the nutrient bath of the isolated rat stomach. He suggests that MAO block increases the potency of those compounds which more easily enter the cells where MAO is active.

The tabulated data show that the molluscan heart frequently gives results which differ strikingly from those obtained with the rat uterus or stomach pre-

parations. This fact should not be overlooked when submitting to bioassay crude extracts from vegetable or animal tissues. The presence, for example, of N-alkylated indolealkylamines would greatly impair the accuracy of estimation of 5-HT.

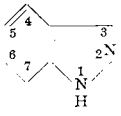

Table 4. *Equipotent molar ratios of indazolealkylamines estimated on four isolated smooth muscle preparations* (BERTACCINI and ZAMBONI 1961). 5-HT taken as unity.

Ring substituent	Side-chain	Stimulant action			
		Rat uterus	*Helix* heart	Rabbit ear	Guinea-pig ileum
5—OH	3—CH_2—CH_2—NH_2	4.3—5[1]	3.9	27	5.3
5—OH	3—CH_2—CH_2—$NHCH(CH_3)_2$	227	172	0	0
5—OC_7H_7	3—CH_2—CH_2—NH_2	0	0	0	0
5—OC_7H_7	3—CH_2—CH_2—$N(CH_3)_2$	0	0	0	0
5—OC_7H_7	3—CH_2—CH_2—$NHCH(CH_3)_2$	0	0	0	0
	3—CH_2—CH_2—NH_2	360	335	134	0
	3—CH_2—CH_2—$N(CH_3)_2$	39	7.2	85	20
	3—CH_2—CH_2—$NHCH(CH_3)_2$	0	0	0	0
	2—CH_2—CH_2—NH_2	0	0	700	570
	1—CH_2—CH_2—NH_2	870	0	66	185

[1] AINSWORTH 1958.

SCHÖTENSACK et al. (1963) have carried out comparative studies on the pharmacological actions of some indolealkylamines and their oxa-analogues. On the blood pressure of rats pretreated with tetraethylammonium, tryptamine and 5-HT were 3 and 500 times, respectively, more potent than the corresponding benzofuranalkylamines; on the rat uterus 10 and 10000 times more active, and on the rat fundus strip 100 and 1000 times more active.

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