Brit. J. Pharmacol. (1955), **10**, 474.

THE USE OF SOME MOLLUSCAN HEARTS FOR THE ESTIMATION OF 5-HYDROXYTRYPTAMINE

BY

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(RECEIVED SEPTEMBER 7, 1955)

This work was undertaken mainly in order to find a sensitive method for the assay of 5-hydroxytryptamine (HT) in tissue extracts. The biological test mostly used has been the contraction of oestrous rat uterus (Erspamer, 1942 ; Amin, Crawford, and Gaddum, 1954), the sensitivity of which lies between 1 and 10 μg./l. This organ is sensitive to other substances, which may be present in tissue extracts, such as adrenaline which inhibits it and oxytocin and bradykinin which stimulate it. Paper chromatographic methods have been described, and that of Jepson and Stevens (1953) detects quantities nearly as small as does the rat uterus, but lack of specificity limits its use.

Erspamer and Ghiretti (1951) demonstrated that enteramine, later identified as HT, stimulated isolated molluscan heart, especially that of *Helix pomatia* and *Octopus vulgaris*. This was confirmed with synthetic HT on octopus heart (Bacq, Fischer, and Ghiretti, 1952). These preparations, however, were not found to be suitable for assay purposes. In the hands of Zetler and Schlosser (1954) *Helix pomatia* heart gave satisfactory results. Welsh (1953) found that *Venus mercenaria* (clam) heart—which had been earlier shown by him (Welsh, 1943) to be suitable for the quantitative estimation of acetylcholine (ACh) in tissue extracts—was stimulated by HT in low concentrations. This heart was used by Twarog and Page (1953) for the determination of HT in tissue extracts. The threshold concentration was 0.2–2 μg./l. and the heart was relatively insensitive to other substances except ACh, the effect of which can, however, be blocked by benzoquinonium (Mytolon) (Ludueña and Brown, 1952), which is used clinically to paralyse voluntary muscles.

Of preparations from different marine invertebrates found on the south coast of Britain, Welsh (1954) suggested the hearts of *Cyprina islandica* and *Buccinum undatum* as the most promising for HT and ACh bioassays. The heart of the freshwater mussel, *Anodonta cygnea*, also reacts to HT with graded responses (Fänge, 1955).

As mentioned above ACh is active on molluscan hearts and the usual response is inhibition. Recently Pilgrim (1954) has studied the action of ACh on the heart of a number of lamellibranch molluscs and Hughes (1955) has described *Mya arenaria* as a sensitive preparation for the assay of ACh. Adrenaline has been reported to stimulate some molluscan hearts in low concentrations, but has often been found quite ineffective. The results of different workers even on the same species have been different. For further information about the pharmacology of the molluscan heart the review of Krijgsman and Divaris (1955) may be consulted. These preparations are sometimes surprisingly different from the vertebrate heart or plain muscle.

This paper describes the actions of certain drugs on the isolated hearts of various molluscs living in the sea near Britain, on the hearts of two snails (*Helix aspersa* and *H. pomatia*), and on preparations of *Lumbricus terrestris*. The heart of the bivalve, *Spisula solida*—formerly known as *Mactra solida*—was found most useful, because of the relative abundance and hardiness of the animal, the constancy of its heart over long periods and its sensitivity to HT. It has the advantage for workers in Great Britain that it is much more easily available that *Venus mercenaria*.

METHODS

In most of the experiments the heart was suspended at room temperature (13–21° C.) in a 2 ml. bath, the contents of which could be changed by overflow, so that the mechanical disturbance was small. The heart of *Buccinum* was cannulated with a Straub cannula through the aorta and the auricle was tied off. Air was always bubbled through the fluid and the movements of the heart were recorded on a smoked drum with a light lever.

Bivalves were opened by cutting both adductor muscles with a thin scalpel. Usually the technique of dissection was essentially that of Welsh and Taub (1948); threads were tied near the auriculo-ventricular junctions and a small piece of ventricle was included in the ligature because of the fragility of the auricles. The modification of this method proposed by Hughes (1955) was occasionally used, but appeared to be less effective when the heart was sluggish.

The ventricles of *Helix aspersa* and *Helix pomatia* were 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 was retained because this seemed to produce a more regular beat.

The crop and gizzard of *Lumbricus terrestris* were suspended together in the same way, and in other experiments strips (2 mm. broad) of longitudinal or circular muscle from the body wall in front of the clitellum were used. The longitudinal strips were taken from the dorsal or ventral midline and all visible nerve chains were removed.

In most of the experiments with marine animals the heart was suspended in a solution of the following composition (g./l.): NaCl 23; Na_2SO_4 4.0; KCl 0.65; $CaCl_2$ 1.1; $MgCl_26H_2O$ 10; $NaHCO_3$ 0.2. Some hearts, which were relaxed and irregular, gave a better beat when the concentration of Mg was reduced 2–5 times. Sometimes at least the sulphate ion seemed to be necessary for regular contractions. Glucose (0.5–1 g./l.) was sometimes also included, but it did not appear to make any difference and was generally excluded. For the heart of *Mya* the solution was that recommended by Welsh and Taub (1948); for *Lumbricus* it was that recommended by Wu (1939), and for *Helix* it was that recommended by Zetler and Schlosser (1954).

To start an inert heart or revive a weak and irregular one the ergot alkaloids, such as ergometrine particularly, have been used (Welsh, 1953; Hughes, 1955). This treatment does not change the sensitivity to acetylcholine, but possibly as a consequence of the increased tonus and the stronger and quicker beat the effects of stimulant drugs such as HT are often obscured.

During preliminary tests in the Gatty Marine Laboratory the molluscs were kept in running sea-water. In this department *Spisula* was kept in cool sea-water (ca. 1 l./animal, changed twice a week) from two weeks to two months. Frequent changes of the sea-water did not seem to increase the lifetime of these animals. Usually the whole supply, collected at one time and place, survived equally long under varying conditions; aeration of the sea-water was not of any obvious advantage. In the same circumstances *Cardium* survived for about the same time. When packed in fresh seaweed and kept in a refrigerator *Spisula*, *Cardium*, and *Mya* survived 1–2 weeks. *Helix* was kept alive in glass jars at room temperature for 5 months or longer.

Drugs were added to the fluid surrounding the heart in the bath and usually left there for 1 min. The interval was dependent on the dose, but the addition of HT, for instance, could usually be repeated as soon as the original beat was again reached. The cycle in HT assays was from 2 to 4 min. unless doses about 100 times larger than the threshold dose were used.

The following drugs were used: bradykinin (M. Rocha e Silva), substance P (Amin and Crawford), 5-benzyloxygramine and 6-methylgramine (Glaxo Laboratories), dihydroergotamine methane sulphonate, lysergic acid diethylamide (LSD), "Hydergine"—a mixture of 3 dihydro ergot alkaloids (Sandoz Products), benzoquinonium chloride (Mytolon), noradrenaline, isoprenaline (Sterling Winthrop Research Institute), histamine acid phosphate, physostigmine sulphate, atropine sulphate, tryptamine hydrochloride, carbachol (The British Drug Houses), hexamethonium bromide (May and Baker), adenosine-5-phosphate (L. Light and Co.), 5-hydroxytryptamine creatinine sulphate (Abbott Laboratories), decamethonium (Allen and Hanburys), acetylcholine chloride (Roche Products), ergometrine maleate, tubocurarine chloride, ouabain (Duncan, Flockhart and Co.), adrenaline, pituitary (posterior lobe) extract (Burroughs Wellcome and Co.), dibenamine (Smith, Kline, and French).

Doses of 5-hydroxytryptamine, tryptamine, adrenaline, noradrenaline, acetylcholine, and histamine are given in terms of the base. Other doses refer to the corresponding salts, although the anion is not mentioned in the text. Doses are in terms of the final concentration in the bath, generally as μg./l.

RESULTS

Experiments with Drug Solutions

The reaction, if any, of the various hearts to HT was always stimulation. Small doses increased the force of the beat and larger doses increased the rate as well. The effect on the force of the beat was generally used to compare different responses. Tryptamine had the same action as HT in concentrations 100 times as great or more (in experiments with *Spisula*, *Cardium*, *Cyprina*, and *Helix*). When the heart was sensitive to ACh the response to small doses was a decrease of the force and rate of the beat. Different salines did not have any clear effect on the response to HT.

Lumbricus terrestris (*Earthworm*)

The crop and gizzard preparation contracted in the presence of HT (10 μg./l.) or ACh (1 μg./l.). It was difficult to obtain graded responses especially with HT, and spontaneous activity had a disturbing effect. The body wall muscles were resistant to HT in concentrations up to 10,000 μg./l.

No satisfactorily beating heart could be obtained from *Barnea candida*, *Mactra corallina*, *Mytilus edulis* (mussel) or *Paphia saxatilis*.

Zirphaea crispata

Graded responses to HT were obtained with concentrations from 1 to 1,000 μg./l.

Solen siliqua (*Razor Shell*)

The threshold concentration of HT for the hearts of *Solen siliqua* was about 1 μg./l., and although they reacted with graded responses with concentrations up to 1,000 μg./l., the three hearts tried were not beating constantly. ACh was active in the same concentrations and ergometrine and LSD stimulated the heart.

Buccinum undatum (*Whelk*)

The cannulated *Buccinum undatum* heart reacted to HT in a concentration of 1 μg./l., but often a dose 100 times higher was ineffective and the responses decreased during the experiment. Welsh (1954) obtained satisfactory results with this preparation, but in our experiments the heart beat was generally irregular and the myocardium was liable to rupture after vigorous beats so that the fluid leaked out and the experiment was spoiled. These animals were difficult to keep alive for more than 1–2 days after transportation to our department.

Cyprina islandica

This shell fish is about 10 cm. long and has a relatively large heart, which beat in the bath with a rate of 10 or less/min. HT and ACh were active in concentrations of 1 μg./l. Noradrenaline and adrenaline stimulated the heart and the threshold concentrations were 1,000 and 5,000 μg./l. respectively. Histamine (5,000 μg./l.) was inactive.

LSD in concentrations from 0.1 to 100 mg./l. increased the amplitude and frequency. Because of the increased amplitude the absolute responses to HT were diminished, but there was no clear evidence of specific antagonism (Fig. 1). The responses to HT and noradrenaline were depressed by the same amount. After benzoquinonium the dose of ACh had to be increased 10 to 50 times for the same response.

Mya arenaria

The threshold dose of HT was often between 0.5 and 2 μg./l., but often 10 or 100 times larger doses were needed. ACh inhibited the heart in concentrations of about 0.1 μg./l. and there were no large differences in the reaction of different animals as with HT. The reaction to carbachol was essentially the same as that to ACh, but the effective dose was 50 times greater. Adrenaline and noradrenaline stimulated, and isoprenaline inhibited, the heart in concentrations of 1,000 μg./l. or higher.

Both ephedrine and cocaine (100 mg./l.) increased the amplitude; in smaller concentrations (0.01–10 mg./l.) they had no action on the response to HT, but sometimes caused a partial block of the effect of adrenaline. Isoprenaline in smaller than threshold doses did not change the responses to HT or adrenaline.

The response to ACh was not definitely changed by physostigmine (10 mg./l.) or atropine (10 mg./l.). Tubocurarine (10–100 mg./l.) depressed it, so that 2–4 times the pretreatment dose was needed to have the same response. The inhibitory effect of isoprenaline was also prevented, but the response to HT remained unchanged.

Hexamethonium and decamethonium in concentrations up to 100 and 10 mg./l. respectively had no effect on the response to ACh or HT.

Histamine stimulated the heart in concentrations of 4 mg./l. or higher. Mepyramine did not prevent this action of histamine, but in concentrations of about 10 mg./l.

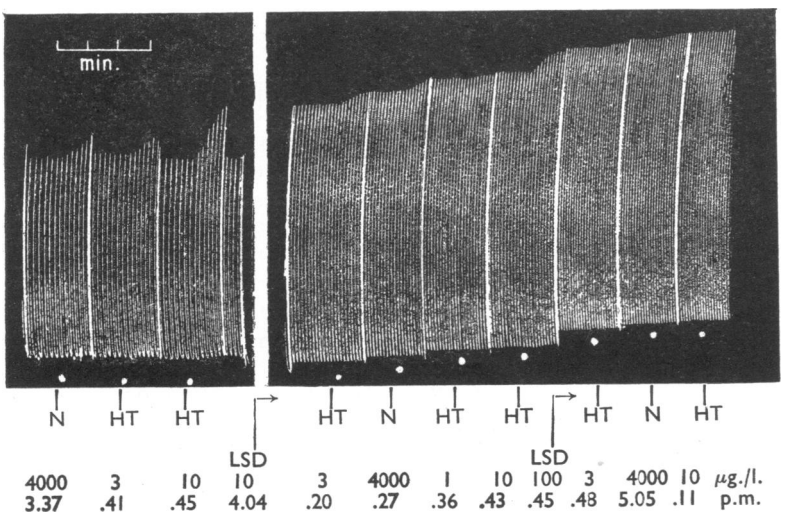

FIG. 1.—Heart of *Cyprina islandica*. 5 ml. bath. Concentrations μg./l. N, noradrenaline; HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide. N and HT in bath for 1 min. LSD in bath continuously.

it had a positive inotropic action, which appeared slowly in 5 min. or more.

Ouabain even in concentrations of 1 g./l. seemed to be without effect. LSD, like other derivatives of ergot, stimulated the heart, but had no specific effect on the response to HT.

The heart was quite sensitive to potassium; an increase of the concentration of KCl from the ordinary 0.9 mg./ml. to 1.15 mg./ml. increased the force and decreased the frequency of the beat. Higher concentrations of KCl decreased the force and finally (2.15 mg./ml.) stopped the heart in diastole, which was followed by slow contraction to systole. The reaction to $CaCl_2$ was usually about the same as to KCl, but the concentration had to be increased in a ratio of 2 or more. The effect of a small increase of KCl could not be prevented by increasing the concentration of $CaCl_2$. Decreasing the concentration of KCl had the opposite actions—decrease in the force and increase in the rate. A decrease of $CaCl_2$ showed the same effect especially clearly. There was no clear change when the concentration of Mg was increased or decreased by 60%.

Cardium edule (*Cockle*)

The heart often beat with a constant amplitude and regular rhythm (8–15/min.), especially on the second day after the dissection. The response to HT was not usually maximal in 2 min., but even when the drug was only left in the bath for 1 min. the threshold concentration was 0.1–1 μg./l. LSD in concentrations of 1–10 μg./l. (left for 1–2 hr.) decreased the amplitude, and only when the heart was already nearly stopped was clear depression of the action of HT seen. 5-Benzyloxygramine and 6-methylgramine in concentrations of 1–10 mg./l. had the same action as LSD, but were 1,000 times less active. The action of these compounds was more or less completely washed off in 2–3 hr. Irregular or inert hearts were not stimulated by ergot alkaloids.

ACh was active in about the same threshold doses as HT. Benzoquinonium in concentrations from 1 to 10 mg./l. blocked about 1 to 100 threshold doses of ACh without having any effect on the response to HT. Atropine (1–10 mg./l.) did not block the response to ACh, and physostigmine (10 mg./l.) had a very weak sensitizing action, if any.

Adrenaline and noradrenaline (100 μg./l. or more) inhibited the heart like ACh (Fig. 2). In the presence of LSD (1–10 μg./l.) 10 times as much adrenaline and noradrenaline or more was needed for a given effect. This blocking effect was only partially abolished after washing until the following day. The action of the adrenaline was not clearly prevented by dibenamine (0.5–6.0 mg./l.) or by the gramines in the above-mentioned doses. Histamine was without effect in concentrations up to 5 mg./l.

Helix aspersa (*Garden Snail*)

The heart was stimulated by HT (*ca.* 1 μg./l.). The maximum reaction was reached in about 20 sec. and graded responses were obtained. LSD, ergometrine, dihydroergotamine, and hydergine all increased the amplitude and could not be shown to block HT. The above-mentioned gramines (1–10 mg./l.) may have had a weak anti-HT action, but they themselves depressed the rate and made the heart-beat irregular.

Adrenaline and histamine (1 mg./l.) were without effect, but ACh inhibited the heart in about the same doses as HT stimulated it. This reaction was not altered by physostigmine, atropine, benzoquinonium, decamethonium, hexamethonium or dibenamine in a concentration of 10 mg./l.

The heart sometimes beat well when superfused and this technique may be useful when increased sensitivity is needed.

Helix pomatia (*Edible Snail*)

The heart beat satisfactorily and reacted like *Helix aspersa* to HT and ACh, the threshold doses being 1 and 10–100 μg./l. respectively. Here again benzoquinonium (10 mg./l.) did not block the response to ACh, and LSD (1–100 μg./l.) and 6-methylgramine (1–50 mg./l.) were not HT-antagonists. Histamine, adrenaline and noradrenaline in concentrations of 5 mg./l. were without an effect.

Spisula (Mactra) solida

This heart started to beat in 5–60 min. after dissection, and the rate was 12–25/min. HT increased the amplitude in concentrations of 0.1–0.5 μg./l. Maximal response was reached within 45 to 60 sec. and relaxation was rapid. Tachyphylaxis was never found. Graded responses were obtained within large dose limits and for the maximum effect the concentration of HT was 1,000 μg./l. (Fig. 3).

LSD (1–10 μg./l.) increased the amplitude and rate and this obscured the action of HT. Ergometrine, hydergine, and dihydroergotamine had the same stimulant action. As Fig. 4 shows, the effect of a brief application of ergometrine was more or less permanent. 5-Benzyloxygramine and 6-methylgramine (Gaddum, Hameed, Hathway, and Stephens, 1955) were tried in doses from 0.1 to 10.0 mg./l. Usually the higher doses made

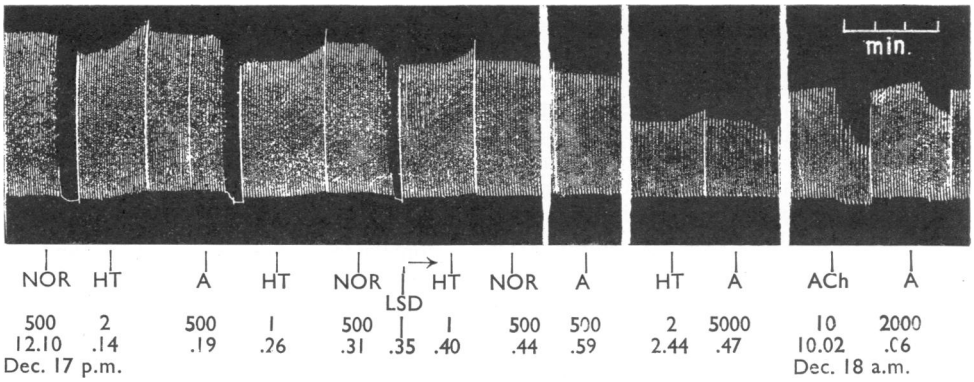

FIG. 2.—Heart of *Cardium edule*. 2 ml. bath. Doses ng. in bath for 1 min. NOR, noradrenaline; A, adrenaline; HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide. 12.35–2.48 LSD 1 μg./l.; 2.48–3.15 LSD 10 μg./l.

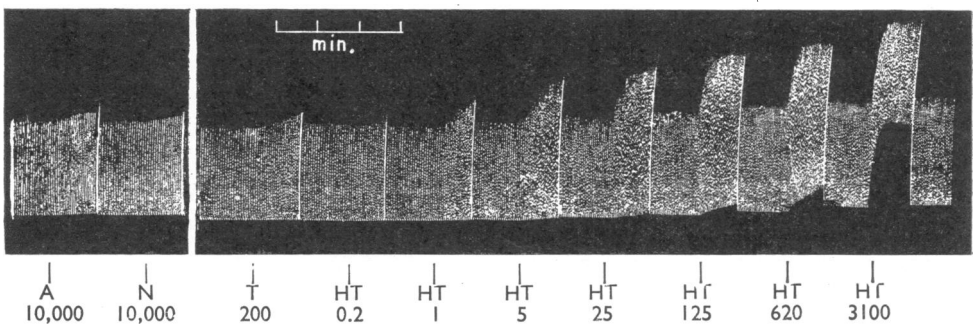

FIG. 3.—Heart of *Spisula solida*. Doses ng. in 2 ml. bath for 1 min. A, adrenaline; N, noradrenaline; T, tryptamine; HT, 5-hydroxytryptamine.

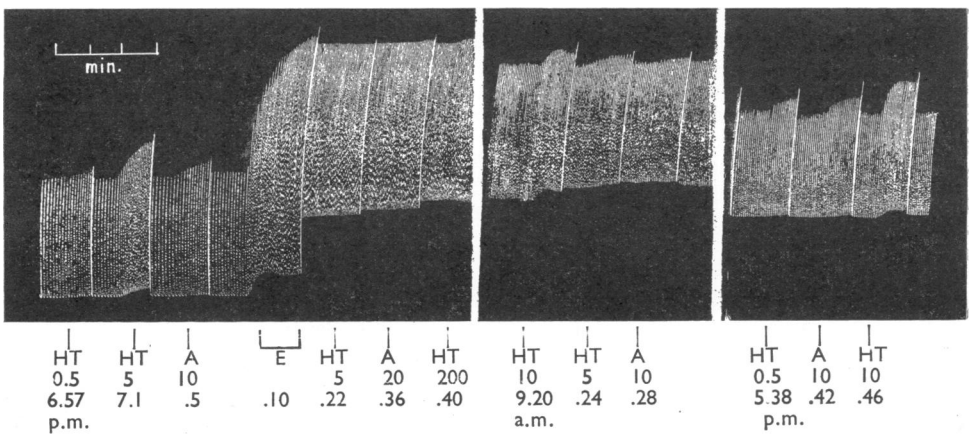

FIG. 4.—Heart of *Spisula solida* in 2 ml. bath. HT, 5-hydroxytryptamine (ng.) A, adrenaline (μg.); E, ergometrine (10 μg. for 90 sec.) increased the tone and rate for more than 24 hr. Responses to both drugs temporarily disappear.

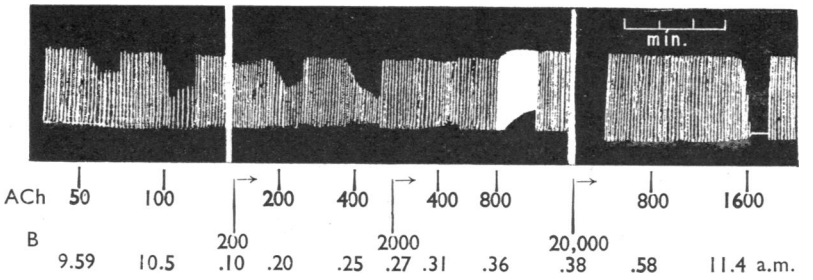

FIG. 5.—Heart of *Spisula solida* in 2 ml. bath. ACh, acetylcholine; B, benzoquinonium. Doses, ng. in bath.

the heart irregular, decreased the rate and increased the amplitude. There was some blocking activity, the highest gramine concentration being able to prevent the effect of 10–50 threshold doses of HT. This action, however, was not specific, the action of adrenaline being depressed as well. The prolonged application of HT itself causes specific densensitization of the guinea-pig ileum (Gaddum, 1953a), but similar experiments with the heart of *Spisula* showed no sign of desensitization.

ACh usually inhibited the heart in threshold concentrations of 1–100 μg./l. Sometimes large doses increased the rate and still larger doses (*ca.* 300 μg./l.) increased the force of the beat with increased tonus before the diastolic arrest. Carbachol had an inhibitory action in ten times higher doses. Physostigmine (10 mg./l.), atropine (10 mg./l.), hexamethonium (25 mg./l.), and decamethonium (25 mg./l.) had no action on the response to ACh. Tubocurarine (50 mg./l. or more) slightly diminished the response to ACh. The only substance found to be an efficient ACh antagonist on the heart of *Spisula* was benzoquinonium (Fig. 5) which is reported to have the same action on the heart of *Venus mercenaria* (Ludueña and Brown, 1952) and *Cyprina islandica* (Welsh, 1954). Benzoquinonium was more effective on hearts that were initially sensitive to ACh and relatively ineffective on resistant hearts. When it was present in concentrations of 1 and 10 mg./l. the threshold concentrations for ACh were about 50 and 300 μg./l. respectively. Adrenaline and noradrenaline stimulated the heart in concentrations of 1,000 μg./l. or more. Histamine in this dose was ineffective, and 0.5 U./ml. or more of pituitary posterior lobe hormones were needed to stimulate the heart. Adenosine, adenosine-5-phosphate, adenosine-diphosphate, and urea had a very small stimulant action in concentrations of 1 g./l. Substance P (P4, activity 18 U./mg., made from horse intestine) had no action in a concentration of 2 U./ml., and there was no reaction to bradykinin (20 μg./ml.) or to the uterus stimulating substance (4 mg./ml.) prepared from urine by Gomes (1955).

When NaH_2PO_4 was added in concentrations of 100–500 mg./l. or more, the amplitude was decreased sometimes after a brief increase.

The addition of 0.0004 N-HCl or more to the bath increased the amplitude, and 0.001 N-NaOH had a small inhibitory action.

Increasing the usual concentration of 0.65 mg./ml. of KCl to 0.9 mg./ml. caused a small increase of amplitude. This effect increased with the dose and at about 2 mg./ml. systolic arrest resulted. The effect of these changes in K concentration on the frequency was not constant.

An increase of the $CaCl_2$ concentration from the usual 1.1 mg./ml. to about 3 mg./ml. usually had a weak negative chronotropic effect, but the response to Ca, even in much higher doses, was not constant. A decrease of the concentration of Ca increased the frequency and decreased the amplitude, and the heart beat well in 0.5 mg./ml. of $CaCl_2$. Sometimes a decrease of Ca may help to make irregular hearts beat at a constant rate.

Experiments with Tissue Extracts

Tissues were extracted with acetone by the method of Amin *et al.* (1954), so that substances soluble in 95% acetone were extracted first, and the residue was extracted with dilute HCl.

The acetone-soluble fraction contains practically all the HT, and Amin *et al.* used it for the estimation of HT with rat uterus. They point out, however, that adrenaline may sometimes be present in sufficient amounts to interfere with the assay by inhibiting the uterus and so masking the effect of HT. Garven (1955) has found that this source of error can be largely eliminated by incubating extracts with the juice which may be pressed out of mushrooms, since this contains an enzyme which destroys adrenaline. In order to get further evidence of the meaning of results obtained in this way the HT-equivalents of acetone extracts of various tissues have been estimated (see Fig. 6) on *Spisula* heart and on the rat uterus, mushroom juice being used with the uterus, but not with the heart.

The results of these assays are shown in Table I. These two methods of estimation usually agreed fairly well with one another, but the values

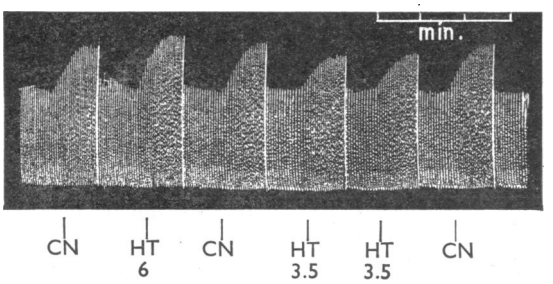

FIG. 6.—Heart of *Spisula solida*. 2 ml. bath. Drugs added every 3 min. and left in for 1 min. Assay of HT (doses in ng.). CN, acetone extract of dog's caudate nucleus. Each dose was equivalent to 21 mg. of tissue and contained less than 6 and more than 3.5 ng.

obtained for the HT-equivalent of extracts of the caudate nucleus with *Spisula* heart were about twice those obtained with rat uterus. Both sets of results are difficult to reconcile with those of Amin *et al.* (1954), who detected no HT in this tissue (<14 ng./g.). The properties of acetone-extracts of the caudate nucleus have therefore been studied in more detail.

TABLE I

THE ESTIMATION OF 5-HYDROXYTRYPTAMINE (NG./G.) ON THE HEART OF *SPISULA SOLIDA* AND RAT'S OESTROUS UTERUS

The values for the first three tissues were obtained in parallel assays. The tissues, except the last two, are from dogs.

	Spisula Heart		Rat Uterus	
Olfactory bulb ..	62, 31, 25	39	40, 33, 43	39
Caudate nucleus ...	88, 200, 60	116	44, 117, 24	62
Cerebellar cortex ..	12, 7, 8	9	<12, <20, <30	0
Sympathetic ganglia	<59, <19, <29	0	<28	0 (<28 bullocks)
Hypothalamus ..	224–526 (6 dogs)	370	220–330 (4 dogs)	280 (Amin *et al.*, 1954)
Human urine ..	100, 89	95 ng./ml.		
Whole *Spisula solida*	706			

The estimates for the first three tissues on rat uterus were by Miss J. Garven.

The results suggest that the acetone-extracts of the caudate nucleus of dogs, cattle, and pigs did in fact contain HT, but that the results were complicated by the presence of other substances.

The solubility of the cardioactive substance in various liquids was similar to that of HT. When extracts were dried on the walls of evaporation flasks about 10% of the activity could be recovered with chloroform, 50% with 99% acetone, and 99% with 95% ethanol. When solutions of HT were treated in the same way similar results were obtained.

On ascending paper chromatograms with the upper alcoholic layer separating from a mixture of 4 vol. *n*-butanol, 1 vol. glacial acetic acid, and 5 vol. water (Partridge, 1948) a substance in extracts acting on both *Spisula* heart and rat uterus was found at the place corresponding to an R_F of 0.4. HT migrated in control strips at the same speed. In both cases the recovery was low (about 25%).

When solutions of HT containing N-NaOH were boiled for 5–10 min. all the activity was lost and similar results were obtained when extracts of dog's caudate nucleus were treated in the same way. The active substance in the caudate nuclei of cattle and pigs seemed to be much more stable and was unaffected by this treatment. This stability was, however, probably due to the presence of protective substances, since HT itself was also stable when added to these extracts. Extracts of the caudate nucleus of dogs did not have this protective action on added HT.

LSD is known to be an active and specific inhibitor of the effects of HT on rat uterus (Gaddum, 1953b). The immediate effects of extracts of the caudate nucleus were also abolished by LSD so that no response was seen in the first minute after the extracts were added to the bath, during which the main response to both the extract and HT had previously occurred. If, however, the extract was left in the bath, a large contraction of the uterus eventually occurred. These results were taken to indicate that the extracts contained HT, and another substance which caused a delayed contraction of the uterus after 1–1.5 min. This substance would not be expected to interfere with the use of rat uterus for the estimation of HT, provided the extracts were not allowed to act for more than 1 min. The main difficulty lies in the fact that estimates of the HT-equivalent of extracts of the caudate nucleus obtained with rat uterus were lower than those obtained with *Spisula* heart (Table I). Similar discrepancies were seen in experiments with acetone extracts of the caudate nuclei of cattle and pigs, in which the HT-equivalent estimated on the rat uterus was 20–80% of that estimated on *Spisula* heart. The results obtained with rat uterus may depend, however, on the doses used, since the log-dose-effect curve for extracts appeared to be flatter than the log-dose-effect curve for HT.

The most probable interpretation of these results is that the extracts contain substances which inhibit the response of the rat uterus to HT. One of these substances may be adrenaline, since Garven (1955) found an increase of the HT equivalent on rat uterus after treatment with mushroom juice, but this treatment was not as completely effective as would be expected if adrenaline were the only inhibitory substance present. If this is the correct explanation, then the assay on *Spisula* heart gives the best indication of the actual amount of HT present.

The dried residue, after extraction with 95% acetone, was then extracted with dilute HCl, as suggested by Amin *et al.* (1954). Such acid extracts of brain, liver, kidney, or urine stimulated the heart of *Spisula* when applied in a concentration corresponding to about 5 mg. of tissue or more in each ml. of bath fluid. This response started sooner and generally lasted longer than the response to HT and was mainly shown as an increase of the rate of the beat. These experiments were disturbed by vigorous foaming, but this was avoided when the extracts were made with 60% acetone. The substance responsible for the foaming then remained undissolved, but extracts still increased the rate of the heart of *Spisula*. The substance causing this effect was insoluble in petrol ether and stable at 100° in N-HCl or N-NaOH. It has not been identified with certainty, but it is possible that the observed effects were due to a mixture of potassium and phosphate.

Discussion

Real sea water is often used to suspend isolated tissues from marine molluscs. Artificial sea waters have been found more satisfactory and this appears to be mainly because the concentration of magnesium in real sea water is too high. This did not seem to matter in the early experiments in the autumn, but later on, in the winter and spring, the use of artificial solutions with a low magnesium content seemed to be more important.

In experiments with *Mya* or *Spisula* the usual effect of a decrease in the concentration of potassium, calcium or magnesium was a quicker and weaker beat and an increase of tone. An increase of the concentrations of these ions often had the opposite effects, but there were complications. For example, the final effect of an increase in the concentration of potassium was generally a slower and more forceful beat, but sometimes, in the autumn, it caused an initial quickening of the beat. We could find no evidence of an antagonism between potassium and calcium on the heart of *Mya* such as has been reported not only for the hearts of vertebrates but also for those of *Anodonta* (Hendrickx, 1945) and *Ostrea* (Jullien, 1931).

Most of these molluscan hearts were stimulated by HT in low concentrations, and by adrenaline or noradrenaline in concentrations about 1,000 times higher. The amounts of adrenaline or noradrenaline in most tissues would not be likely to interfere with the use of these hearts for the estimation of HT. Several of the species studied could be used for assays, but the best results were obtained with *Spisula solida*. The heart is rather small and some practice is required to set it up, but once it started beating it usually continued to give regular results for long periods and could sometimes be used for three days. The animals were usually easy to obtain and survived for reasonable periods in the laboratory. The effect of HT developed rapidly and disappeared rapidly when the solution was changed, so that the solutions to be tested could be applied at intervals of 2–4 min., except when very large doses were used. With low doses the time interval could be varied widely without affecting the result.

Some of the other species were not much studied because of the weakness of the muscle, or because it seemed to be difficult to obtain regular supplies of the animal, or because they did not survive long in captivity.

We have not found a really satisfactory specific antagonist for HT on molluscan heart. Welsh (1953) tried a few indoles, but they were excitatory themselves or ineffective. LSD, which is an effective antagonist for HT on rat uterus (Gaddum, 1953b), is said by Welsh (1954) to be also effective on the hearts of *Cyprina, Buccinum,* and *Venus.* In our experiments on the hearts of *Cyprina, Cardium,* and *Spisula* the antagonism has appeared to be unspecific, and feeble except when the heart was maximally stimulated by the LSD (Fig. 1). Other ergot alkaloids, including ergometrine, also stimulated the hearts of various molluscs and obscured the effects of HT and adrenaline (Fig. 4). Ergometrine has little or no action against HT on rat's uterus or perfused rabbit's ear. The stimulant action of these ergot alkaloids on the molluscan heart lasts for many hours after a brief application of the drug, and use has been made of this fact in assays of ACh on the hearts of *Venus* and *Mya* (Welsh and Taub, 1948 ; Hughes, 1955).

The inhibitory action of ACh on molluscan hearts forms the basis of well-known tests for this

substance. The heart of *Spisula* was often much less sensitive to ACh than those of certain other species and would probably not be especially suitable for the assay of this substance.

Physostigmine had no definite effect on the response to ACh and this confirmed the conclusion of most previous workers with other species (Bacq and Coppée, 1937; Welsh and Taub, 1948; Smith and Glick, 1939). All agree that atropine does not antagonize ACh on molluscan heart (Prosser, 1940); some say, however, that it paralyses the inhibitory nerves (Hendrickx, 1945). The only active antagonist of ACh is benzoquinonium. In *Venus mercenaria* this substance was effective in equimolar doses with ACh (Ludueña and Brown, 1952). In *Venus* Welsh (1953) found that benzoquinonium blocked the inhibition of the heart produced by stimulation of the visceral ganglion, and he mentions (Welsh, 1954) that this substance antagonizes ACh also on the isolated heart of *Cyprina islandica* and *Buccinum undatum*.

Benzoquinonium antagonized ACh in our experiments with *Spisula*, but high activity was only found when the hearts were initially very sensitive to ACh. The sensitivity of the heart in the presence of a given concentration of benzoquinonium appeared to be fairly constant, and, if the hearts were initially insensitive, the benzoquinonium had little effect. In some insensitive *Spisula* hearts there was only feeble blockage of the temporary stimulation and tonus increase after large doses of ACh even in a concentration of 10 mg./l. of benzoquinonium (Dose ratio = 3–4). The ACh receptors in pulmonates seem to be different from those in lamellibranchs. In *Helix aspersa* and *H. pomatia* benzoquinonium did not antagonize ACh nor did any other of the drugs tested. This fact limits the usefulness of *Helix* hearts for the assay of HT.

The heart of *Cardium edule* often gave regular results over long periods. HT and ACh in low concentrations had the same actions on this heart as they had on other hearts, but the actions of adrenaline, noradrenaline and the ergot alkaloids were unexpected. Adrenaline and noradrenaline caused inhibition even in concentrations which had no action on other species. The effect of LSD was also inhibition instead of stimulation, and was followed by a period in which the inhibitory effect of adrenaline and noradrenaline was absent (Fig. 2).

Histamine had no effect except in high doses, which sometimes stimulated or depressed the heart. Mepyramine did not antagonize the action of histamine on the heart of *Mya*, but itself stimulated this heart.

When HT and ACh are both present in a solution each may interfere with the estimation of the other. As Welsh (1954) has pointed out, it is therefore desirable to use antagonists to make the assay more specific. In experiments on HT, benzoquinonium may be used to exclude the effects of ACh, and in experiments on ACh LSD may be used to exclude the effects of HT. Since the effect of LSD may be feeble and unspecific it is particularly desirable in the latter case to determine by direct experiment how much HT may be present in the solutions to be tested without affecting the result.

Summary

1. The effects of various drugs on isolated hearts from a dozen species of molluscs have been studied.

2. The heart of *Spisula solida* provides a convenient method of estimating 5-hydroxytryptamine (HT) which generally stimulates it in a concentration of 0.1–1 μg./l. It is not sensitive to other known substances in tissue extracts, except acetylcholine, the effects of which can be abolished with benzoquinonium. The animal is hardy and its heart beats constantly for several days in isolation.

3. Estimates obtained in this way of the concentration of HT in some tissues agree with those obtained with rat uterus. A discrepancy was observed with extracts of the caudate nucleus and this is attributed to the presence of unknown pharmacologically active substances.

4. The heart of *Cardium edule* is inhibited by adrenaline.

5. Benzoquinonium antagonizes the effect of acetylcholine on the hearts of bivalves but not that on the hearts of snails.

We are especially grateful to Dr. J. M. Dodd, who arranged for one of us (M.K.P.) to work in the Gatty Marine Biological Station, St. Andrews, for a few weeks in the early stages of this investigation. We are indebted to the following for providing animals: The Scottish Marine Biological Association, Marine Station, Millport, Isle of Cumbrae; the Marine Biology Station, Menai Bridge, Anglesey; the Freshwater Biological Association, Windermere Laboratory, The Ferry House, Far Sawrey, Ambleside; and Professor G. A. H. Buttle.

The work was made possible by a grant to M. K. Paasonen from the Finnish Government and to J. H. Gaddum from the Medical Research Council, London.

REFERENCES

Amin, A. H., Crawford, T. B. B., and Gaddum, J. H. (1954). *J. Physiol.*, **126**, 596.

Bacq, Z. M., and Coppée, G. (1937). *Arch. int. Physiol.*, **45**, 310.

—— Fischer, P., and Ghiretti, F. (1952). Ibid., **60**, 165.

Erspamer, V. (1942). *Arch. exp. Path. Pharmak.*, **200**, 43.

—— and Ghiretti, F. (1951). *J. Physiol.*, **115**, 470.

Fänge, R. (1955). *Experientia*, **11**, 156.

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

—— (1953b). Ibid., **121**, 15P.

—— Hameed, K. A., Hathway, D. E., and Stephens, F. F. (1955). *Quart. J. exp. Physiol.*, **40**, 49.

Garven, J. D. (1955). Ph.D. thesis, Edinburgh.

Gomes, F. P. (1955). *Brit. J. Pharmacol.*, **10**, 200.

Hendrickx, J. P. (1945). *Arch. int. Pharmacodyn.*, **71**, 214.

Hughes, B. (1955). *Brit. J. Pharmacol.*, **10**, 36.

Jepson, J. P., and Stevens, B. J. (1953). *Nature, Lond.*, **172**, 772.

Jullien, A. (1931). *C. R. Soc. Biol., Paris*, **108**, 77.

Krijgsman, B. J., and Divaris, G. A. (1955). *Biol. Rev.*, **30**, 1.

Ludueña, F. P., and Brown, T. C., Jr. (1952). *J. Pharmacol.*, **105**, 232.

Partridge, S. M. (1948). *Biochem. J.*, **42**, 238.

Pilgrim, R. L. C. (1954). *J. Physiol.*, **125**, 208.

Prosser, C. L. (1940). *Biol. Bull. Wood's Hole*, **78**, 92.

Smith, C. C., and Glick, D. (1939). Ibid., **77**, 321.

Twarog, B. M., and Page, I. H. (1953). *Amer. J. Physiol.*, **175**, 157.

Welsh, J. H. (1943). *J. Neurophysiol.*, **6**, 329.

—— (1953). *Arch. exp. Path. Pharmak.*, **219**, 23.

—— (1954). *Nature, Lond.*, **173**, 955.

—— and Taub, R. (1948). *Biol. Bull. Wood's Hole*, **95**, 346.

Wu, K. S. (1939). *J. exp. Biol.*, **16**, 184.

Zetler, G., and Schlosser, L. (1954). *Arch. exp. Path. Pharmak.*, **222**, 345.