single equation given by Thiesen *et al.* (*30*) for 0° to 42°C, and of the values from three cubic equations used by Chappuis (*21*) to cover the ranges 0° to 10.3°, 10.3° to 13°, and 13° to 41°C. Thus these data had already been smoothed on the assumption that they were free from kinks. Had Qurashi gone directly to the equations, he would have found discontinuities only at the change of function in Chappuis's work; other kinks are computational artifacts.

The pattern common to all reports that we have examined, including those that we have not discussed in detail, is that the size of the supposed discontinuity is comparable to the degree of accuracy of the measurement. Experimenters commonly overestimate their degree of accuracy (*31*), and errors often produce odd points that do not fit a smooth plot. Accordingly, we believe that the discontinuities so far reported in the properties of liquid water are artifacts. The wide scatter of the temperatures at which discontinuities have been reported strongly supports this conclusion. The balance of evidence is that the physical properties of water *do* vary continuously with temperature.

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Species Specific Effect of Acetylcholine on Bivalve Rectums

Abstract. *The pharmacology of acetylcholine and 5-hydroxytryptamine on the rectums of* Katelysia rhytiphora *and* K. scalarina *was found to be similar, in general, to that of other bivalves. However, while acetylcholine causes a fast twitch in the rectum of* K. rhytiphora, *the activity and tone of the* K. scalarina *rectum are depressed except at high concentrations of the drug. The two species can be distinguished by these responses, and, therefore, these rectums are useful experimental objects for studying the physiology of molluscan visceral muscle.*

Animal physiologists often need to compare the results of similar experiments performed on different species of animals (*1*, *2*). The usual assumption is that, if the species are sufficiently closely related and if the system under consideration is sufficiently complex, then the probability that differences are based on species is small. This report describes some relatively simple pharmacological responses of homologous tissues from two sympatric intrageneric species.

Acetylcholine (ACh) has three observed effects on the rectum of the clam *Mercenaria mercenaria*: depression of tone and rhythmic activity, phasic contraction, and tonic contraction (*1*). These effects are dependent on the dose of ACh and the tone

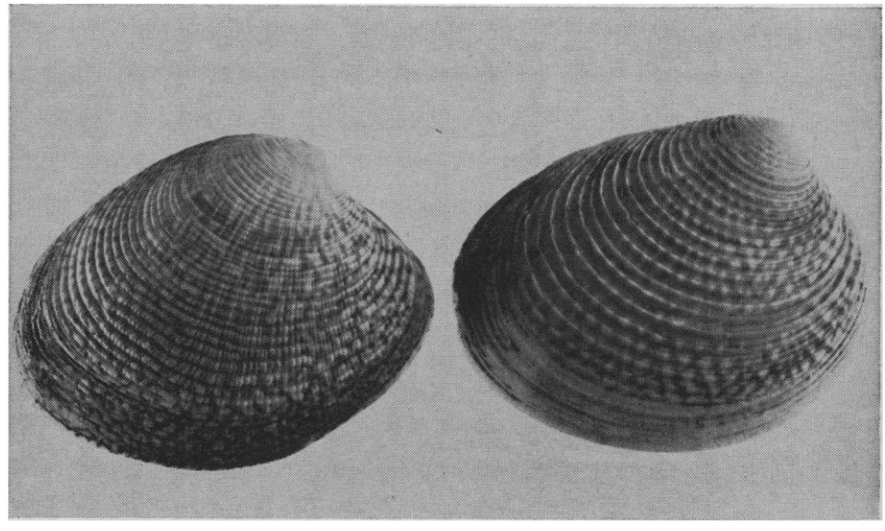

Fig. 1. *Katelysia rhytiphora* (left) is distinguished by the anastomosing concentric ridges and by the fine striations radiating from the umbo. *K. scalarina* (right) has stronger, uninterrupted, smooth concentric ridges and no radiating striations (*6*). Both specimens are right valves coming from animals collected together at Blairgowrie, Victoria, Australia. Enlarged × 1.4.

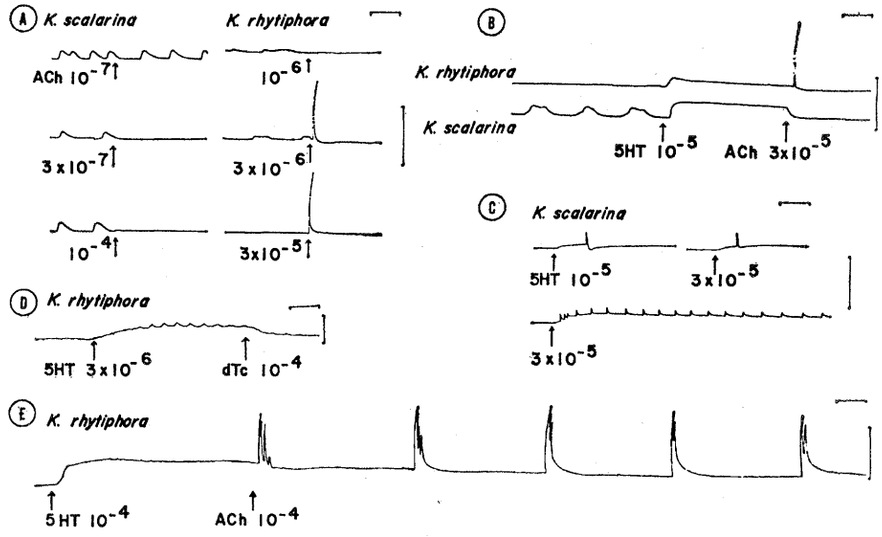

Fig. 2. Effect of acetylcholine (ACh) and 5-hydroxytryptamine (5HT) on the rectums of the bivalves *Katelysia scalarina* and *K. rhytiphora*. (A) Comparison of the action of ACh on the two species. (B) Addition of $3 \times 10^{-5}M$ ACh to the bath after treatment with $10^{-5}M$ 5HT. (C) Effects of 5HT on two preparations of *K. scalarina*. One rectum, lower line, responded with a series of small phasic contractions superimposed on a tonic contraction. (D) Inhibition of 5HT excitation by *d*-tubocurarine (dTc) in *K. rhytiphora*. (E) Effect on *K. rhytiphora* of ACh following a previous dose of 5HT. All doses were given at the times indicated by arrows and represent molar concentrations in the organ bath. Time marker is 1 minute; the force marker is 200 mg.

of the muscle preparation. The same pattern of responses occurs in the rectums of other species of mollusks, although it may vary qualitatively with species (*2*). For example, in *Mercenaria* the phasic response is usually small, and frequently it is absent. Recently, Prosser, Nystrom, and Nagai (*3*) demonstrated in the *Spisula solidissima* rectum that, while the tonic response was similar to that in *Mercenaria*, the phasic contraction was much more prominent and always occurred. In *Busycon canaliculatum*, a gastropod, they observed only a phasic response.

The three rectal responses to ACh were sought in two species of the bivalve genus *Katelysia*. *Katelysia rhytiphora* Lamy, 1935 and *K. scalarina* Lamarck, 1818 are venerids of similar size (3 to 5 cm) that live buried in the sand between the intertidal zone and about a meter below low water. Their distribution along the southern coast of Australia is similar. The animals I used came from the beach between Blairgowrie and Rosebud on Port Phillip Bay, Victoria. They were obtained from beds of eel grass (*Zostera*), where they could often be found growing side by side. Since the sexual cycles of the two species are similar (*4*), the experimental animals were probably in the same reproductive stage. These species were discussed in detail by Nielsen (*4*), who also showed that their isolation is due to the incompatibility of their gametes. The distinguishing features of the shells are shown in Fig. 1.

In these experiments, the segment of rectum that runs through the ventricle was always used; dissection and preparation of this tissue have been described elsewhere (*1*). Tension changes were recorded with a force transducer and ink-writing oscillograph. The perfusion fluid was filtered sea water, and temperature of the bath was maintained between 15° and 16°C throughout the experiments. Doses are expressed as the final concentration of the drug in the bath. Usually, two rectums, one from each species, were run simultaneously, so that temperature, perfusion fluid, and time between doses were identical for the two preparations.

Dominant response of the *K. rhytiphora* rectum to ACh is a phasic twitch with a contraction time of about 1/10 second and a half relaxation time of about 2 seconds (Fig. 2A). There may be two or more twitches in a single response (Fig. 2E). Threshold for the phasic contraction is between 10^{-6} and $3 \times 10^{-6}M$ ACh. At this concentration, and below it ($3 \times 10^{-7}M$), a small depression of muscle tone was also observed. In some preparations, especially at high concentrations of ACh, there was also a tonic excitor component which began about halfway down the phasic relaxation and which faded with a time constant of 2 or 3 seconds.

The *K. scalarina* rectum, on the other hand, never showed a phasic response to ACh (Fig. 2A). At low doses ($10^{-7}M$ ACh) activity of a spontaneously active rectum was decreased. At $10^{-6}M$ the rectums became quiescent, and the tone was also decreased. Even at high doses ($10^{-4}M$ ACh) depression was predominant, although a small tonic contraction often appeared.

5-Hydroxytryptamine (5HT) raised the tone of the rectum and induced rhythmical activity in both species. The effect was most striking in *K. scalarina* (Fig. 2C); in two preparations, 5HT (3×10^{-6} to $10^{-4}M$) induced twitches similar to although smaller than those observed in *K. rhytiphora* after treatment with ACh. These twitches can be distinguished from much smaller phasic contractions which also appeared and which were characteristic of the effect of 5HT on *K. rhytiphora* (Fig. 2D).

The effect of ACh, after treatment with 5HT, remained unaltered in both species (Fig. 2B). Activity of the rectum was reduced in *K. scalarina*. In *K. rhytiphora* no depression was seen; the same ACh twitch appeared and, in one experiment, a high dose of ACh ($10^{-4}M$) produced a series of twitches following excitation with $10^{-4}M$ 5HT (Fig. 2E). In both species, effects of 5HT on the rectum were antagonized (Fig. 2D) by *d*-tubocurarine and by benzoquinonium chloride (*9*). Similar results have been reported for *M. mercenaria* (*1*) and *Tapes watlingi* (*2*).

These two closely related species of *Katelysia* can, without error, be distinguished by the responses of their rectums to ACh. With less certainty, the effects of 5HT are also diagnostic. Since the physical characteristics of the animals, their habitats, and the conditions of the experiments were similar, the observed pharmacological differences are probably solely species dependent.

How can the pharmacological differences be explained? Three approaches to this problem are possible: (i) by way of model systems based on pharmacological data from similar prepa-

rations; (ii) by reference to electrophysiological findings on bivalve visceral muscle; and (iii) by consideration of comparative anatomy.

The general pattern of the pharmacology of the *Katelysia* rectum is similar to that of *Mercenaria mercenaria* (*1*), *Tapes watlingi* (*2*), and other clams [*Anodonta grandis, Mercenaria campechiensis, Macrocallista nimbosa, Mytilus edulis,* and *Mytilus planulatus* (*5*)]. The depressor and excitor actions of ACh, and especially the blockade of 5HT by anticholinergic agents, were the crucial pharmacological findings upon which Greenberg and Jegla (*1*) and Phillis (*2*) proposed their model control systems for the clam rectum. The models differed in detail, but both included the possibility of excitation and inhibition by ACh at specific cholinergic sites in neuromuscular junctions. Both allow for excitation by 5HT either at sites on the muscle (*1*) or by stimulation of excitor cholinergic fibers (*1, 2*). Depression by 5HT occurred in *Mercenaria* and was explained by stimulation of depressor neurons (*1*). A 5HT depressor component is also built into the *Tapes* model. A feature of both models is that the observed response to any dose of 5HT or ACh is the sum of at least two antagonistic components. Consequently, the magnitude and direction of the observed response depends upon the dose and relative effectiveness of the drug at the antagonistic receptors. The relative smallness of the tonic excitation by ACh and the large persistent depressor effect on the rectum of *K. scalarina* could be due to a greater sensitivity to ACh at the depressor neuromuscular junction than at the excitor. Furthermore, this relatively large depressor effectiveness must be greater than that for *K. rhytiphora.* It is only by even more complex and unjustifiable theoretical contortions that the phasic response of the *K. scalarina* rectum to 5HT, but not to ACh, can be explained by these models.

Neither model can explain the phasic response to ACh. Greenberg and Jegla (*1*), on pharmacological evidence, suggested different mechanisms for the tonic and phasic responses of *M. mercenaria.* Prosser *et al.* (*3*) were able to produce these effects, not only by application of ACh, but also by direct electrical stimulation of the rectum and by indirect stimulation through the visceral ganglion. They were also able to separate the tonic and phasic responses: a spike preceded the phasic response but not the tonic; the tonic and phasic excitation properties were different; and the phasic, but not the tonic, contraction was abolished in high concentrations of potassium. They suggest, for the rectum of *Spisula solidissima,* not only a tonic excitor and depressor, but also a separate phasic excitor innervation. It is not likely that *K. rhytiphora* would have this phasic innervation while *K. scalarina* does not.

The phasic contraction, and its associated spike, is probably a function of the synchrony of contraction of muscle cells in the rectum. One would expect that such structural features as density and distribution of connective tissue, extent and type of contact between muscle cells, and density and type of innervation might be dissimilar in the rectums of the two species. Nielsen (*4*) found the histology of the entire alimentary tracts of the two species, as well as the gross anatomy of the nervous systems, to be similar.

However, a further study, restricted to the rectum and using ultramicroscopic (*7*) and established histochemical (*8*) techniques, might reveal a structural basis for the pharmacological differences.

The rectums of other pairs of intrageneric species of bivalves have been examined [*Mercenaria mercenaria* and *M. campechiensis*; *Mytilus edulis* and *M. planulatus* (*5*)]; in neither case were there discernible differences in pharmacology. However, the two sympatric species of *Katelysia* have minimal evolutionary variability, and also show very different physiological properties. Therefore, they are potential and unique tools for the investigation of these properties.

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7. See N. C. R. Merrillees, G. Burnstock, M. Holman, *J. Cell Biol.* **19**, 529 (1963).
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9. Benzoquinonium chloride (Mytolon) was provided by A. M. Lands, Sterling-Winthrop Research Institute.
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Histamine Synthesis in Man: Inhibition by 4-Bromo-3-Hydroxybenzyloxyamine

Abstract. *Oral administration of 4-bromo-3-hydroxybenzyloxyamine to normal humans resulted in decreased urinary excretion of histamine; the normal increase in urinary levels of histamine after oral histidine loading was prevented. In two patients having systemic mastocytosis, additional evidence of inhibition of biosynthesis of histamine included marked reduction in symptoms attributed to histamine, and prevention of symptomatic exacerbation associated with histidine loading.*

Histamine is a naturally occurring amine that possesses a wide range of potent pharmacologic effects. Investigators have proposed important roles for this substance in the mediation of various physiologic functions (*1*) and in the pathogenesis of many human diseases (*2*). Thus a drug that effectively inhibits the biosynthesis of histamine in man should be of interest both as a tool for physiologic investigations and as a potential therapeutic agent.

Histamine is synthesized in mammalian tissues by decarboxylation of the precursor amino acid, histidine; its biosynthesis differs from that of other biogenic amines, such as norepinephrine and serotonin, in at least two important respects: firstly, decarboxylation, being the only enzymic process involved, is rate-limiting; secondly, at least in the rat, biosynthesis of histamine is catalyzed by a specific histidine decarboxylase (*3*). Thus inhibition of histidine-decarboxylase activity may be expected to result in more or less spe-