lems associated with the presence of similar compounds (*3*) in commercially cultivated plants led us to examine the ornamental wood roses, *Ipomoea tuberosa* L. and *Argyreia nervosa*, both common Hawaiian crops that have assumed commercial importance as components of dried tropical flower industry.

Seeds of *I. tuberosa*, *A. nervosa*, and seeds of the *I. purpurea* cultivars, "Pearly Gates" and "Heavenly Blue," were purchased locally. The seed powder was wetted with NH_4OH and extracted with ether (*2*), the solvent was removed, the residue was dissolved in dilute sulfuric acid, the acid solution was extracted with ether and neutralized, and the alkaloids were extracted with chloroform. Portions of this chloroform extract were assayed for alkaloids by the addition of a modified Erlich's dimethylaminobenzaldehyde reagent containing ferric chloride (van Urk's reagent) (Table 1). Examination of dried sepals of *I. tuberosa* showed a trace amount of alkaloid while comparable sepals from *A. nervosa* were devoid of substances giving a positive reaction with van Urk's reagent.

Portions of the chloroform extracts were also subjected to thin-layer chromatography on silica gel (*4*). A number of fluorescent zones (which responded to van Urk's reagent) were detected in the extracts from morning glories and the small wood rose (Table 2). The major components separated from each extract were eluted, and the quantity of alkaloid was determined with van Urk's reagent. Portions of the eluates from preparative thin-layer chromatography were subjected to paper chromatography with butanol–acetic acid–water (4:1:1) as the developing solvent. The material at R_F 0.24 on thin layer was resolved into two substances with R_F's of 0.61 and 0.70, respectively, identical with those of authentic specimens of isoergine and penniclavine. The other zones from the thin-layer chromatography appeared to be homogeneous.

The principal alkaloids found in "Pearly Gates" by Taber *et al.* (*2*) were ergine, isoergine, and penniclavine, and these appear to be the principal alkaloidal constituents in "Heavenly Blue" and *A. nervosa* seed.

The seed of *A. nervosa* is the best plant source of ergoline alkaloids discovered; it contains approximately 3 mg of alkaloidal material per gram of seed. Approximately one-eighth of this is lysergamide. Since the small wood rose is easily and commonly cultivated it appears to be a most useful tool for studying the biosynthesis of these substances in plants.

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Serotonin and Adenosine Triphosphate: Synergistic Effect on the Beat Frequency of Cilia of Mussel Gills

Abstract. *For each tenfold increase in the concentration of serotonin in the range 10^{-7} to 10^{-4}*M, *the beat frequency of the lateral cilia of the gills of the freshwater mussel* Elliptio complanatus *increases by approximately two beats per second over the mean frequency of 14.5 beats per second for control gills perfused with 0.04*M *potassium chloride. The addition of 10^{-6} to 10^{-3}*M *concentrations of adenosine triphosphate has no detectable effect on the beat frequency. The addition of both serotonin and 10^{-4}*M *adenosine triphosphate increases the frequency by two beats per second more than does the addition of serotonin alone.*

It has been hypothesized that serotonin (5-hydroxytryptamine, 5HT) functions as a local cilioregulatory hormone in the lamellibranch gill (*1, 2*). Endogenous 5HT, as well as 5-hydroxytryptophan decarboxylase activity, has been demonstrated in gills of several mussels including those of *Mytilus*, *Modiolus*, and the freshwater genus *Anodonta*. The lateral cilia of the gills of these mussels were found to respond to physiological concentrations of 5HT by a prompt and reversible increase in frequency.

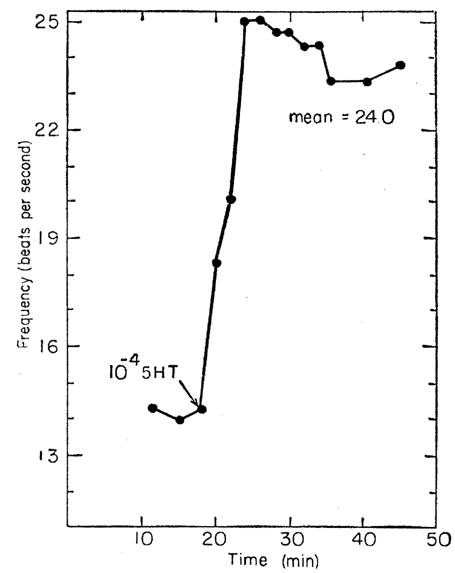

Fig. 1. The effect of serotonin on the beat frequency of lateral cilia. At 18 minutes (arrow), 5HT is added to the KCl perfusion fluid, giving a new mean equilibrium frequency within 3 minutes.

Adenosine triphosphate (ATP) has been previously implicated in ciliary contraction mechanisms as well as certain processes involving serotonin. Born (*3*) found that platelets showed increased uptake of 5HT in the presence of ATP and that the ATP and 5HT were probably stoichiometrically bound. Deyrup (*4*) found that cilia of the frog pharyngeal epithelium respond to physiological concentrations of ATP by a prompt and reversible increase in beat frequency. Adenosine triphosphate acts as an energy source in ciliary contraction. Previous workers have demonstrated the ability of ATP to activate glycerine-extracted models of cilia and flagella (*5*). I have investigated the possibility of a further connection between 5HT and ATP in the control of ciliary beat.

Small stripped pieces of gill tissue from the freshwater mussel *Elliptio complanatus* were placed in a perfusion chamber for continuous microscopic and stroboscopic examination, as first described by Satir (*6*). This allowed continuous observation of the gill tissue while it was subjected to rapid and reproducible changes in exogenous concentrations of 5HT, and made it possible to obtain precise kinetic data. Beat-frequency determinations were made by noting the frequency of the stroboscopic illumination at which the metachronal wave appeared to remain stationary.

Figure 1 shows a curve obtained during a typical experiment and illustrates the method used to determine the "mean frequency at equilibrium." The gill tissue was perfused with $0.04M$ KCl at zero time until the lateral cilia showed good metachronism (*6*). Three determinations of frequency (after 12, 16, and 18 minutes) were made in the KCl perfusion bath. At 18 minutes the perfusion fluid was changed to $0.04M$ KCl plus $10^{-4}M$ 5HT (as creatinine sulfate salt). A rapid increase in beat-frequency was immediately apparent, the frequency reaching a maximum value after 3 minutes, after which many frequency determinations could be made for filaments selected randomly over the surface of the gill. The mean of the values thus obtained was the mean frequency at equilibrium for the lateral cilia at the specified concentration of 5HT. Thus a mean value of 24.0 beats per second was obtained in the experiment illustrated in Fig. 1. Frequencies remained low in control preparations to which no 5HT was added.

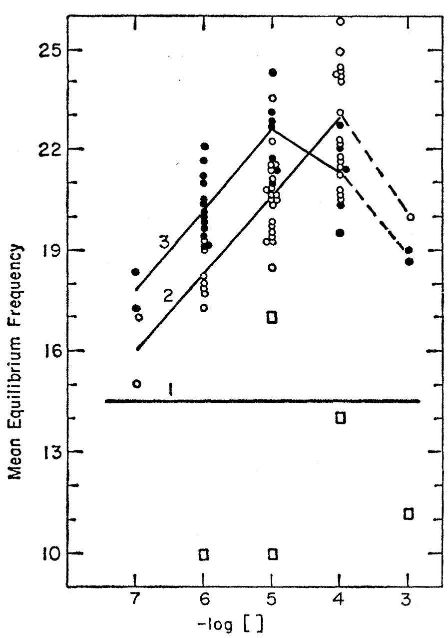

Fig. 2. Summary of frequency responses of cilia to varying exogenous concentrations of ATP or 5HT. Baseline (curve 1) represents mean value of $0.04M$ KCl. Addition of varying concentrations of ATP to the perfusion fluid causes little or no increase in beat frequency (squares), while addition of 5HT (curve 2, open circles) at concentrations above $10^{-7}M$ increases frequency. The addition of low concentrations of 5HT together with $10^{-4}M$ ATP (curve 3, black circles) causes the beat frequency to increase more than when 5HT is added alone. The overall effect is a displacement of the curve for the 5HT response to the left.

Table 1. Representative experiments demonstrating the response of the lateral cilia to various concentrations of exogenous 5HT or ATP, or both, added to the $0.04M$ KCl perfusion fluid.

Experiment	Addition to perfusion fluid	Mean frequency at equilibrium (beats per sec)	
		Initial	After addition
	*Initial perfusion fluid containing 0.04*M *KCl alone*		
A	$10^{-5}M$ ATP	16.9	17.1
B	$10^{-3}M$ ATP	13.0	11.3
C	$10^{-7}M$ 5HT	14.5	17.0
D	$10^{-6}M$ 5HT	14.6	19.0
E	$10^{-5}M$ 5HT	16.8	20.4
F	$10^{-4}M$ 5HT	10.5	24.4
G	$10^{-4}M$ ATP + $10^{-7}M$ 5HT	16.7	18.4
H	$10^{-4}M$ ATP + $10^{-6}M$ 5HT	13.3	19.7
I	$10^{-4}M$ ATP + $10^{-5}M$ 5HT	16.6	23.2
	*Initial perfusion fluid containing 0.04*M *KCl + 10^{-5}*M *5HT*		
J	$10^{-4}M$ ATP	20.5	22.7
	*Initial perfusion fluid containing 0.04*M *KCl + 10^{-6}*M *5HT*		
K	$10^{-4}M$ ATP	19.3	21.2

The results shown in Table 1 indicate that there were great individual variations in the mean equilibrium frequencies for cilia perfused with $0.04M$ KCl alone. Values as high as 17 and as low as 9 beats per second were obtained during the course of the study. Most of the values ranged between 13 and 16, giving a mean value of 14.5 beats per second for cilia in the KCl bath. This is expressed graphically in curve 1 of Fig. 2, which then serves as a base line.

Mean equilibrium values for various concentrations of ATP (squares) are shown in Fig. 2. The values obtained with ATP are not significantly different at the .05 level of probability from the values obtained with KCl alone (Table 2) (*7*). When 5HT was added to the KCl perfusion fluid, the beat frequency increased by approximately 2 beats per second for each tenfold increase in the concentration of 5HT in the range 10^{-7} to $10^{-4}M$ (Fig. 2, curve 2).

The addition of $10^{-4}M$ ATP to the KCl perfusion bath containing 5HT gave unexpectedly high results (Table 1; Fig. 2, curve 3). Each concentration of 5HT in the presence of $10^{-4}M$ ATP resulted in a mean frequency at equilibrium corresponding to that obtained only with a tenfold higher concentration of 5HT in the absence of ATP; the difference in frequency was approximately two beats per second. The paired data from experiments with 10^{-6} to $10^{-5}M$ 5HT alone against 5HT plus $10^{-4}M$ ATP are significantly different at the .05 level of probability (Table 2). The synergistic effect of ATP and 5HT is thus apparent in this range.

Table 2. Probability values for data of Fig. 2. [*P*] represents the probability that two groups give the same population of points.

Paired groups	[*P*]
$10^{-6}M$ 5HT versus $10^{-6}M$ 5HT + ATP	0.0002
$10^{-5}M$ 5HT versus $10^{-5}M$ 5HT + ATP	.002
$10^{-4}M$ 5HT versus $10^{-4}M$ 5HT + ATP	.03
KCl versus ATP	.074

Possible explanations of this observation are: (i) 5HT and ATP act indirectly by way of the nerves below the gill epithelium, (ii) the presence of exogenous ATP facilitates the uptake of 5HT by the cilia, and (iii) the ATP used in the lateral cilia is contained within membrane-limited structures (*8*) and is thus available only at a limited rate. When the membrane is destroyed or interrupted, as in glycerinated models of cilia, 5HT is ineffective and ATP increases frequency directly. In light of Deyrup's report of the effects of ATP on the pharyngeal epithelium of the frog, it is interesting to note that this same tissue is insensitive to 5HT (*2*).

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