cient to kill a significant proportion of the population. Where the specificity of the agent is known, the results presented in Table 1 are in accord with the above explanation. Alkylating agents (dimethyl and diethyl sulfate, methyl methanesulfonate, nitrogen mustard, imines, epoxides, and propiolactone) are known to alkylate the nitrogen in the 7 position in guanine, selectively (*13*). The most significant effect of hydroxylamine treatment is transition of a cytosine-guanine base pair to an adenine-thymine base pair (*14*). Exposure of DNA to nitrous acid results in deamination of the bases; the rate of deamination is fastest with guanine, intermediate with cytosine, and slowest with adenine (*15*). The sensitivity of bacteria to x-radiation (and hence probably to γ-radiation) is directly proportional to cytosine-guanine of their DNA (*16*). It is unfortunate that the chemical specificity of NFT and MNG are unknown. If this explanation for selective bleaching is correct, then these compounds should affect primarily the adenine or thymine bases in DNA. Finally, if chloroplasts should lack a mechanism for repairing the damage in chloroplast DNA, any lesions would be permanent; thus, although the growth rate of a treated population of *E. gracilis* returns to normal after temporary inhibition, the ability to form chloroplasts is irreparably lost. Lack of such a repair process cannot be the primary reason for the selectivity of certain radiomimetic agents except in the event that the lesions introduced by bleaching agents are refractory while those caused by nonbleaching radiomimetic agents can be repaired.

Although selective bleaching by MNG and nitrofuran derivatives and lack of such bleaching by many other mutagenic agents can be rationalized, it is uncertain whether this explanation can serve in the case of other chemical bleaching agents. Streptomycin, a powerful bleaching agent, reacts with DNA in vitro (*17*) and in virus particles (*18*) and increases the frequency of mutations affecting host range in phage T_2 grown in sensitive bacteria (*19*). However, recent work with bacteria indicates that streptomycin probably acts by binding to ribosomes, which causes a "mis-reading" of the messenger RNA code during protein synthesis (*20*). Schere and Collinge (*21*) have suggested that the bleaching action of streptomycin can be explained on this basis if it is assumed that the protein synthesizing system of the chloroplast is more sensitive than that of the cytoplasm. Errors in chloroplast proteins would thus lead to inactivation of the ability to form chloroplasts.

D. R. McCalla

Research Unit in Biochemistry, Biophysics and Molecular Biology, McMaster University, Hamilton, Ontario, Canada

References and Notes

1. J. L. Jinks, *Extrachromosomal Inheritance* (Prentice-Hall, Englewood Cliffs, N.J., 1964).
2. See D. S. Ray and P. C. Hanawalt, *J. Mol. Biol.* **9**, 812 (1964) for references.
3. See D. R. McCalla, *J. Protozool.* **12**, 34 (1965).
4. H. Lyman, H. T. Epstein, J. A. Schiff, *Biochim. Biophys. Acta* **50**, 301 (1961).
5. A. Gibor and S. Granick, *J. Cell Biol.* **15**, 599 (1962).
6. J. K. Setlow, *Photochem. Photobiol.* **3**, 393 (1963).
7. A. Zamperi and J. Greenberg, *Biochem. Biophys. Res. Commun.* **14**, 172 (1964); D. R. McCalla, *Can. J. Biochem. Physiol* **42**, 1245 (1964); *Can. J. Microbiol.* **11**, 185 (1965).
8. S. Aaronson and S. Schere, *J. Protozool.* **7**, 156 (1960).
9. J. J. Wolken, *Euglena: An Experimental Organism for Biochemical and Biophysical Studies* (Institute of Microbiology, Rutgers Univ., New Brunswick, N.J., 1961).
10. J. O. Hutchens and B. Podolsky, *J. Cellular Comp. Physiol.* **43**, 205 (1954).
11. R. B. Setlow, *ibid.* **64**, Suppl. 1, 51 (1964).
12. A. Gibor and S. Granick, *Science* **145**, 890 (1964).
13. P. Brookes and P. D. Lawley, *J. Cellular Comp. Physiol.* **64**, Suppl. 1, 111 (1964); F. Dickens, *Brit. Med. Bull.* **20**, 96 (1964).
14. E. Freese, E. Bautz, E. B. Freese, *Proc. Nat. Acad. Sci. U.S.* **47**, 845 (1961).
15. H. Schuster, *Biochem. Biophys. Res. Commun.* **2**, 320 (1960).
16. H. S. Kaplan and R. Zavarine, *ibid.* **8**, 432 (1962).
17. S. S. Cohen, *J. Biol. Chem.* **168**, 511 (1947).
18. T. D. Brock and S. O. Wolley, *Science* **141**, 1065 (1963).
19. B. Fernandez, F. L. Haas, O. Wyss, *Proc. Nat. Acad. Sci. U.S.* **39**, 1052 (1953).
20. J. Davis, W. Gilbert, L. Gorini, *ibid.* **51**, 883 (1964).
21. S. Schere and J. C. Collinge, *Plant Physiol.* **39**, Suppl. 34 (1964).
22. I thank the following for gifts of chemicals: Cancer Chemotherapy National Service Center, NIH for azaserine; Merck Sharp and Dohme for nitrogen mustard; and American Cyanamid for triethylene melamine. MNG was purchased from Aldrich Chemical Co. and crystallized twice from ethanol; butadiene diepoxide was purchased from the California Corporation for Biochemical Research, and mitomycin C from Sigma Chemical Co. For the remaining agents, the purest grade obtainable was used without further purification. I thank Olja Eelnurme for technical assistance. Financial assistance from the National Research Council of Canada is gratefully acknowledged.

15 January 1965

Ergoline Alkaloids in Tropical Wood Roses

Abstract. *Extracts of* Argyreia nervosa, *a tropical wood rose, contain appreciable quantities of ergoline alkaloids tentatively identified as ergine isoergine, and penniclavine together with related substances.*

The discovery by Hofmann (*1*) that some members of *Convovulaceae* contain ergoline alkaloids has stimulated investigation of other plants in this family. Taber *et al.* (*2*) examined the seeds of 16 ornamental morning glories and detected similar substances in 13 of these cultivars. Among the compounds tentatively identified as being present was the known psychotomimetic isoergine (lysergamide).

The possible health and legal prob-

Table 1. Alkaloid contents of fresh seeds.*

Variety	Average seed		Yield (mg alkaloid/g)†	
	Wt (g)	Vol (ml)	Present work	Taber *et al.*
		I. purpurea		
Heavenly Blue	0.037	0.02	0.813	0.24
Pearly Gates	0.039	0.03	0.423	0.42
		I. tuberosa L.		
	1.400	1.15	Nil	
		A. nervosa		
	0.109	0.11	3.050	

* Expressed as ergonovine maleate equivalents. † Average of three or more distinct seed samples.

Table 2. Contents of major alkaloids in three plants by thin-layer chromatography.

R_F	Yield per gram of fresh seed*			Product†
	Pearly Gates (μg)	Heavenly Blue (μg)	*A. nervosa* (μg)	
0.15				
.24	78	219	555	Isoergine & Penniclavine
.29				Ergometrine
.45				Ergometrinine
.56	69	81	780	Ergine

* Expressed as ergonovine maleate equivalents. † Tentative identification.

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.

JOHN W. HYLIN
DONALD P. WATSON
Hawaii Agricultural Experiment Station, University of Hawaii, Honolulu

References and Notes

1. A. Hofmann and A. Tscherter, *Experientia* **16**, 414 (1960); A. Hofmann and A. Cerletti, *Deut. Med. Wochschr.* **86**, 885 (1961); A. Hofmann, *Planta Med.* **9**, 354 (1961).
2. W. A. Taber, L. C. Vining, R. A. Heacock, *Phytochemistry* **2**, 65 (1963).
3. N. Gordon, *Reporter* **29**, 35 (15 Aug. 1963).
4. M. Klavehn and H. Rochelmeyer, *Deut. Apotheker-Ztg.* **101**, 477 (1961).
5. Published with the approval of the Director of the Hawaii Agricultural Experiment Station, University of Hawaii, Technical Note No. 12.

4 December 1964

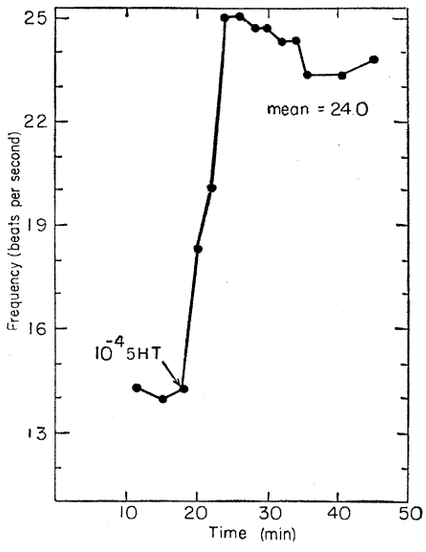

Fig. 1. The effect of serotonin on the beat frequency of lateral cilia. At 18 minutes (arrow), 5HT is added to the KCl perfusion fluid, giving a new mean equilibrium frequency within 3 minutes.

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.

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.