surface of the cell has not been ascertained; it may protect against ultraviolet radiation, since more deeply pigmented races are generally more resistant to ultraviolet than less deeply pigmented ones (*3*).

Appearance of an albino mutant *B. intermedium* of the Rao-A stock made it possible to compare the resistance to radiation of pigmented and albino clones. Greater resistance in the pigmented than in the albino clone would suggest that the pigment is a screen protecting the cell from short-wavelength ultraviolet radiation. Conversely, less resistance in the pigmented than in the albino clone would suggest that in the colored clone the pigment acts as a photosensitizer to ultraviolet, as it does to intense visible light.

The albino and red clones of the Rao-A stock were both grown on a single strain of bacteria in continuous yellow light at 25°C. Individuals in the same nutritive condition were tested with various dosages of low-intensity (about 4 to 8 erg mm^{-2} sec^{-1}) ultraviolet light at wavelength 2654 Å from a monochromator (*4*). The rate of regeneration, a sensitive indicator of the action of ultraviolet radiation (*4*), was used to compare effects on the two clones. Posthypostomal pieces of pigmented *Blepharisma* at 25°C recover their mouth parts and form food vacuoles within about 5 hours (5.3 ± 0.3 hr) after removal of the hypostome; radiation prolongs the regeneration time (*4*).

Times for 50-percent regeneration of the pigmented and albino clones after various ultraviolet dosages (*5*) are compared in Fig. 1. The albino clone is not only much more sensitive to radiation than the pigmented clone but is also the most susceptible *Blepharisma* ever tested (*3*). Thus it appears somewhat a paradox that the very pigment that photosensitizes pigmented *Blepharisma* to intense visible light apparently acts as a protective screen against short-wavelength radiation. None of the data presented, however, exclude the possibility that the mutation from pigmented to albino may be accompanied by a loss in a repair mechanism; such loss, rather than lack of a screening pigment, might explain the decreased resistance of the albino.

Electron-microscope studies of pigmented and albino clones (isolated from an unnamed species of *Blepharisma* but subsequently lost) indicate the presence of similar granules in both—with possibly some modification in the albino (*6*). It is therefore likely that the mutation from pigmented to albino form involves a gene that determines an enzyme concerned with synthesis of the pigment but not necessarily with elimination of the granules. The albino clone, the subject of this report, has not yet been studied with the electron microscope.

The pigment of *Blepharisma* absorbs visible as well as ultraviolet light and is bleached by the latter (*7*). In ponds it may serve as a protective screen against solar ultraviolet, perhaps enabling *Blepharisma* to occupy otherwise untenable habitats. Little is known of the ecology of *Blepharisma* (*8*).

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Cycasin: Radiomimetic Effect

Abstract. *Cycasin, methylazoxy-methanol-β-glucoside, a carcinogenic compound that occurs naturally in cycad plants, induces chromosome aberrations in the root-tip cells of* Allium cepa. *Germination and growth in an aqueous solution of cycasin at a concentration of only 3 percent of that found in* Cycas circinalis *induced as many aberrations in chromosomes as did about 200 roentgens of gamma irradiation.*

It has been demonstrated that parts of the cycad plant and the compound cycasin isolated from tissue of *Cycas circinalis* L. are acutely toxic and induce neoplasms in experimental animals (*1, 2*). Cycasin is methylazoxymethanol-β-glucoside (*3*). Several "neocycasins" having the same aglycone but different sugar moieties have been recognized (*4*).

It has long been known that various agents can produce radiomimetic and carcinogenic effects (*5*). We tested cycasin (*6*) for radiomimetic effects on plant chromosomes, as measured by the induction of aberrations in chromosomes.

An aqueous solution of cycasin, 5 mg in 10 ml, was poured over several layers of filter paper in a petri dish and 100 onion seeds (*Allium cepa* var. Dowing Yellow Globe) (*7*) were sown on the paper. Other seeds sown on filter paper moistened with water served as controls; some of the controls were given an acute dose of 260 r of gamma rays when the root tips were about 0.2 cm long. Some root tips from each batch were fixed in a mixture of alcohol and acetic acid when the roots were about 0.7 cm long; others were fixed the following day when the roots were about 1.5 cm long. Acetocarmine smears were made for cytological analysis. Chromosome aberrations (Table 1), scored at anaphase, included dot deletions, rod deletions, and chromatid and chromosome bridges.

Aberrations found in the controls result from natural aging of the seeds. As seedling roots increase in length the aberrations, caused by aging or by acute radiation, decrease in frequency

Table 1. Numbers of chromosome aberrations induced in cells of onion root tips by germination and growth in cycasin solution or by gamma irradiation.

Root length, fixed (cm)	Cells scored (No.)	Aberrations (No.)	
		Total	Per 100 cells
Onions grown in water (controls)			
0.7	426	17	4
1.5	416	12	3
Onions grown in cycasin solution			
0.7	898	90	10
1.5	352	116	33
Roots irradiated (260 r) after growth in water			
0.7	802	353	44

because cells with aberrations are less likely to continue mitosis, but the decrease in frequency of aberration in control roots fixed when about 1.5 cm long is not statistically significant. The longer roots grown in the cycasin solution had a significantly higher frequency of aberration than the shorter roots; this may result from the longer duration of treatment or from differential sensitivity during the nuclear cycle. In the longer root tips the cycasin produced about as many aberrations as irradiation of 0.2-cm roots with 200 r of gamma rays.

It has been postulated that the carcinogenic quality of cycasin may relate to action of the aglycone as an alkylating agent in forming diazomethane in vivo (*8*). Evidence from germfree animals and injection experiments indicates that it is probably the unstable aglycone rather than cycasin itself that is the active toxic and carcinogenic agent (*1, 3*). Many plants are known to contain emulsins of the type that split cycasin. It may be that cycasin affects *Allium* chromosomes by way of its slow hydrolysis to form methylazoxymethanol.

The finding that cycasin induces breaks in *Allium* chromosomes raises questions concerning both the nature of the resistance of cycad plants to cycasin and the radiomimetic effects of other carcinogenic agents.

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Circadian Rhythm in Pineal Serotonin: Effect of Monoamine Oxidase Inhibition and Reserpine

Abstract. *The pineal gland of the rat shows a circadian rhythm in its serotonin content, the amount of serotonin decreasing at night. This decrease can be prevented by inhibiting the action of monoamine oxidase. Reserpine abolishes the circadian rhythm in pineal serotonin in the same manner as does interruption of the sympathetic nervous connections of the central nervous system and the pineal gland. These observations suggest that circadian changes in release and binding of serotonin may occur in the pineal gland, and that a central mechanism in which monoamines participate may control the circadian pineal-serotonin rhythm.*

There are at least two circadian rhythms in the pineal gland of the rat; one is concerned with the enzymatic synthesis of melatonin (*1*), a gonadal inhibitory hormone (*2*), and another, with the pineal content of serotonin, a precursor of melatonin (*3*). The activity of hydroxy-indole-*O*-methyl transferase, the enzyme which synthesizes melatonin, is highest at midnight and lowest at 6 p.m. (*1*). This rhythm is completely exogenous and can be extinguished by blinding the animals or by changing their environmental lighting. The concentration of serotonin in the rat pineal gland varies from a maximum of 60 to 80 ng per milligram (wet weight) at 1 p.m. to a minimum of 10 to 30 ng per milligram at 11 p.m. This rhythm is endogenous and persists for at least 2 weeks in blinded animals and in rats kept in continuous darkness (*4*). The nocturnal fall in the serotonin content of the pineal gland can be prevented by allowing the lights to remain on for an additional 4 hours on a given day (*4*). Both the melatonin (*1*) and serotonin rhythms (*4, 5*) are abolished by severing the sympathetic nerves to the pineal gland.

Several mechanisms could cause the marked rise and fall in the serotonin content of the pineal gland. More serotonin might be formed during the day than at night, or more might be destroyed at night. It is also possible that changes occur in the binding and release of serotonin during the day and night.

We estimated the activity in the pineal gland of 5-hydroxytryptophan decarboxylase, the enzyme which forms serotonin, and of monoamine oxidase, an enzyme which metabolizes serotonin, every 4 hours during a 24-hour period, using groups of 10 rats for each time-point. In this and subsequent experiments Sprague-Dawley female rats (180 to 200 g) were kept in a room at 25°C and were subjected to cycles of 14 hours of light (fluorescent lighting was automatically turned on at 5 a.m.) and 10 hours of darkness (lights turned off at 7 p.m.) for at least 1 week. The activities of 5-hydroxytryptophan decarboxylase (*6*) and monoamine oxidase (*7*) were measured by specific and sensitive methods. There were no changes in either monoamine oxidase or 5-hydroxytryptophan decarboxylase activities during the day or night.

In other experiments we examined the possibility that changes in the synthesis of serotonin in vivo, or in the transport of amino acid precursors into intracellular synthetic sites in the pineal gland, might account for the circadian changes in the serotonin content of the pineal gland. Groups of ten rats each received intraperitoneal injections of 5-hydroxytryptophan (100 mg/kg) or tryptophan (200 mg/kg) at noon or at 10 p.m. and the serotonin content of single pineal glands was measured 1 hour later by a fluorometric method specific for serotonin (*8*). The increment in pineal serotonin content in rats injected with tryptophan (100 ng/mg) and in rats injected with 5-hydroxytryptophan (60 ng/mg) was the same at night and during the day. These experiments suggest that the changes in the serotonin content of the pineal gland are not the consequence of changes in synthesis of this biogenic amine.

Since there are large amounts of serotonin (*3*) and monoamine oxidase (*9*) in the pineal gland and since both serotonin (*10*) and monoamine oxidase (*11*) are highly localized in sympathetic nerve endings in the pineal gland, it seems probable that serotonin is bound and stored in the gland in such a form as to be inaccessible to destruction by monoamine oxidase. Quay (*3*) observed that the nocturnal fall in pineal serotonin is precipitous. He found that between 7 p.m., when the lights were turned off, and 11 p.m. the serotonin content of the pineal gland decreased at a rate of 25 ng per gland per hour to about 20 percent of its value of 7 p.m. The rapid nocturnal decline of