Pt(II) (Pt to DNA base input ratio of 2.3×10^{-3}) for 1 hour at 37°C as shown in Fig. 2 and then with 0.1*M* thiourea for 1 hour at 37°C. Instead of 99.6 percent inactivation (0.4 percent survival; closed circles in Fig. 3) inactivation was decreased to 98.0 percent (2 percent survival; open circles in Fig. 3). Since 2 percent survival is still considerably less than the 12 percent survival found after 18 hours in 1*M* thiourea, this result shows that the predominant effect of prolonged incubation with 1*M* thiourea is to reverse the lethal lesions present in the λ DNA, and not merely to prevent their formation.

This study shows that thiourea reverses Pt(II)-induced DNA cross-links and lethal lesions in isolated DNA. Since *cis*-Pt(II) is a useful anticancer agent, it is a question of considerable interest whether thiourea or a related compound can reverse cytotoxic lesions of the drug in vivo. Burchenal *et al.* (*8*) have recently reported that thiourea can block the action of *cis*-Pt(II) complexes on mouse leukemia cells. It remains to be determined whether toxic lesions produced by Pt complexes in mammalian cells can actually be reversed. This question can be approached in the context of recent studies of the kinetics of formation and removal of DNA-related cross-links in mammalian cells treated with pharmacologically reasonable doses of Pt complexes (*9*).

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Melatonin Synthesis by the Retina

Abstract. *Melatonin fulfills many of the criteria for classification as a hormone. Until recently it was considered to be elaborated exclusively by pineal organs. Melatonin synthesis by other tissues has been indicated but not demonstrated unequivocally. Trout retinas in a whole-organ culture system in vitro synthesized tritiated melatonin from a substrate containing tritiated serotonin. This raises the possibility that the trout retina is an endocrine organ.*

Several sources of evidence indicate that tissues other than the pineal gland are capable of melatonin formation. However, an exacting demonstration of nonpineal melatonin synthesis has not, to our knowledge, been accomplished. Three tissues, lateral eye retina (*1–3*), enterochromaffin cells (*4, 5*), and Harderian gland parenchyma (*1, 2*) contain melatonin, but it has not been shown that the melatonin is synthesized by these tissues rather than obtained by uptake from a circulating pool of melatonin originating from the pineal. Hydroxyindole-*O*-methyltransferase (HIOMT), the enzyme that catalyzes the *O*-methylation of *N*-acetylserotonin, which results in the formation of melatonin, has been demonstrated in the retina (*6–8*) and Harderian gland (*9*). Nevertheless, HIOMT activity, although being indicative of melatonin formation, does not irrefutably imply this molecule's synthesis, because it can catalyze the formation of several compounds in addition to melatonin (*10*).

Attempts have been made to establish the rat retina as a melatonin-synthesizing tissue both in vivo and in vitro by demonstrating the conversion of isotopically labeled precursor to melatonin (*11, 12*). However, with the techniques then available, it was not possible to demonstrate conclusively that melatonin synthesis occurs in this tissue.

By using whole-organ cultures of rainbow trout (*Salmo gairdneri*) retinas, we have shown that the retina is capable of synthesizing melatonin from a serotonin substrate.

Individual, whole retinas, stripped of all pigmented epithelium, were incubated in a medium of Hanks buffered saline solution, containing glucose, $1 \times 10^{-6}M$ norepinephrine, and [^{3}H]serotonin hydrochloride (70,000 count/min; 2.39 ng) under an atmosphere of 95 percent O_2 and 5 percent CO_2 for 4 hours at 21°C. After incubation, each retina was homogenized in its incubation medium and the resulting homogenate extracted (*13*). Each retinal extract (50 μl) was spotted separately onto a thin-layer chromatography (TLC) plate that was subsequently developed in a solution of chloroform and methanol (9:1). Upon development, the ascent lanes were cut into 20 segments (0.05 R_F each) and the synthesized ^{3}H-labeled molecules deposited along the ascent lanes were quantified by liquid scintillation spectrometry with toluene, PPO-POPOP (*14*), and Triton X as the fluors.

A ^{3}H-labeled molecule with a mobility identical to melatonin (R_F 0.50 to 0.65) (*15*) was formed by the incubated retinas (Fig. 1). No ^{3}H-labeled products were found on the TLC plates on which extracted medium blanks containing no tissue were spotted. However, melatonin and 5-methoxytryptamine cannot be separated easily by TLC procedures (*16*) and 5-methoxytryptamine can be formed by *O*-methylating serotonin (HIOMT catalyzed) (*10*); therefore, melatonin synthesis, although suggested, was not conclusively demonstrated.

Individual retinas were cultured as before and extracts of each retina and medium were subjected to the same TLC separation procedure. This time, however, the entire area of the ascent lane where melatonin was suspected to reside, after development of the chromatogram, was removed and the tritiated products were eluted off the lanes with chloroform. The combined eluates were dried under N_2 and then resuspended in 200 μl of phosphate-buffered saline and 0.1 percent gelatin (PBS-gel). After allowing 24 hours for resuspension, we reextracted this suspension in 2.5 ml of petroleum ether (*17*). Melatonin-specific rabbit antiserum in EDTA-PBS (2.5 μl of a 1:400 initial dilution) was added to a 100-μl portion of the petroleum ether–extracted, resuspended retinal extract, resulting in a final dilution of 1:16,000 of the specific antibody. Another 100-μl portion was removed and 2.5 μl of normal rabbit serum in EDTA-PBS (1:400 initial dilution, 1:16,000 final dilution) was added. These suspensions were incubated at 4°C for 24 hours in a shaker bath. The petroleum ether eluate was taken to a volume of 200 μl under N_2, after which four 50-μl portions were removed and placed into scintillation vials. The amount of ^{3}H-labeled product removed by this extraction was then quantified.

After the 24-hour incubation period, the ^{3}H-labeled products bound to normal rabbit serum and the [^{3}H]melatonin bound to the specific antibody were as-

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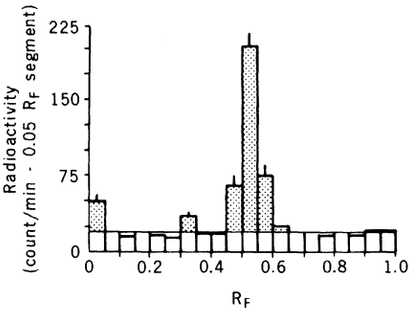

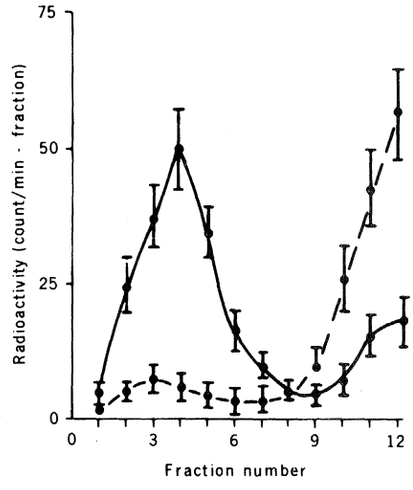

Fig. 1 (left). Chromatogram of the homogenized, extracted retinas and medium. The area of greatest radioactivity, where melatonin was expected to reside (*15*), was from R_F 0.50 to 0.65. The TLC plate (Eastman 6060 silica gel G) was developed in a mixture of chloroform and methanol (9:1). Fig. 2 (right). Radioactivity associated with successive fractions collected from two columns of Sephadex G-25 gel (6.0 by 0.5 cm; equilibrated with EDTA-PBS buffer) on which resuspended retinal extracts incubated with either melatonin-specific rabbit antiserum (—) or normal rabbit serum (---) had been placed. Fractions from the column separating components of retinal extract incubated with melatonin-specific antibody contained more radioactivity in the excluded volume than fractions from the column separating the retinal extract incubated with normal rabbit serum.

sessed by passing the retinal extracts through columns of Sephedex G-25. The tritiated products were quantified by liquid scintillation spectrometry (Fig. 2).

Significantly more ^{3}H-labeled molecules were collected in the void volume from the extract incubated with melatonin antibody than in the void volume from extract incubated with normal rabbit serum. This melatonin-specific antibody has a very low affinity for 5-methoxytryptamine and other indole derivatives (*15*). Therefore, our results, obtained by a variety of techniques, indicate that one of the ^{3}H-labeled products formed during the incubation of retinas with labeled precursor is authentic melatonin. Only 0.6 percent of the radioactivity in the total retinal extract was removed by petroleum ether extraction. This probably represents normal extraction loss.

The photoreceptor cell component of the retina is the probable site of synthesis of melatonin within the retina (*1*), but the functional significance of retinal melatonin is unknown. Radioimmunoassay of trout retinas demonstrates that the concentration of melatonin is high during the photophase and low during the scotophase (*18*), confirming that the rhythm in retinal melatonin production is antiphasic when compared to that demonstrated for the plasma of trout (*19–21*) and other animals (*2, 15, 22–24*) or that measured in the pineal gland of trout (*19*). Because of this "reversed" phasing, it seems unlikely that retinal melatonin participates in regulating the migration of the pigmented epithelium which protects retinal photosensory components from intense illumination. However, this does not preclude the possibility that pineal melatonin may have a role in this phenomenon (*25*), although rabbit retinas do not actively accumulate melatonin from the medium in vitro (*26*).

Because the site of synthesis is probably the photoreceptor, one adaptive role for retinal melatonin may be in mechanoresponsiveness of photoreceptor outer segments to bright light. It is known that lower vertebrate photoreceptors are retracted during periods of bright illumination (*27*) and that melatonin facilitates this event (*28*). Perhaps high daytime titers of endogenous retinal melatonin are involved in this response.

Our experiments demonstrate that the retina is capable of melatonin synthesis (*29*) and thus undermine the commonly held belief that pinealectomy may be equated with total melatonin removal. We also have evidence that the trout retina releases melatonin into the blood (*30*). Furthermore, if nonpineal, melatonin-synthesizing tissues undergo a compensatory increase in melatonin secretion after pinealectomy (*23, 31*), then experiments on the effect of pinealectomy as a means of melatonin removal may be equivocal. If the trout retina is responsible for a portion of the circulating melatonin titer, and if the evidence supporting melatonin as a hormone is accepted, then the trout retina could be considered as an endocrine organ.

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13. Retinas were suspended in 0.5 ml of phosphate-buffered saline and 0.1 percent gelatin (PBS-gel) and homogenized in an ice-cooled glass tissue grinder. The homogenates were taken to a volume of 1.0 ml in PBS-gel, then extracted in 5.0 ml of analytical grade chloroform. The extract was sequentially washed in sodium bicarbonate buffer (*p*H 10.25) and deionized water. The washed extract was then reduced to a volume of 50 μl under N_2 gas. This procedure has an extraction efficiency for melatonin of approximately 80 percent.
14. The abbreviations PPO and POPOP are for 2,5-diphenyloxazole and 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene, respectively.
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29. Using the same whole-organ culture technique coupled with the extraction and chromatography procedure described here on gerbil Harderian glands, we have found that these glands also synthesize a ^{3}H-labeled product with the exact mobility as [^{3}H]melatonin on our TLC system.
30. Eighteen bilaterally enucleated trout had significantly lower plasma concentrations of melatonin (102.5 ± 5.9 pg/ml; $P < .05$) than 22 intact fish (128.3 ± 5.3 pg/ml). Twenty-one fish with sham operations had 118.2 ± 5.4 pg of melatonin per milliliter of plasma; this value was not significantly different from either the blinded or intact animals (W. A. Gern and C. L. Ralph, in preparation).
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