in concentrations of a few nanograms per milliliter. Note that the assay does not distinguish between Mt or thionein.
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Phototherapy-Induced Hypocalcemia in Newborn Rats: Prevention by Melatonin

Abstract. *When young rats are exposed to white fluorescent light the concentration of calcium in their serum decreases. This effect is prevented by shielding the occiput, by inhibiting corticosterone synthesis, and by exogenous melatonin. Furthermore, the expected hypocalcemic response to cortisol injection is prevented by melatonin. Light-induced hypocalcemia may result from increased calcium uptake by bone when the blocking effect of melatonin decreases after pineal inhibition by transcranial illumination.*

Jaundice is a common problem in newborn infants, and the risk of brain damage from the cytotoxic effects of bilirubin justifies efforts to prevent or ameliorate excessive concentrations of bilirubin in the plasma. Exposure to intense light (phototherapy) accelerates the formation of photobilirubin, a metastable geometric isomer efficiently excreted in bile. Approximately 2.5 percent of all babies born in the United States are so treated (*1*). Both white and blue fluorescent lights are used (*2*).

Premature infants undergoing phototherapy with white light have an increased incidence of hypocalcemia compared to coeval controls under standard nursery conditions (*3*). Blue light, though effective in reducing hyperbilirubinemia, produces no significant change in serum calcium (*4*). These findings suggest that phototherapy-induced hypocalcemia is not mediated by bilirubin metabolites. Since all infants wear eye shields during phototherapy, the hypocalcemic effect of light probably involves extraoptic pathways. We have studied this phenomenon in newborn rats, and our results suggest that white light affects calcium homeostasis by inhibiting pineal secretion of melatonin.

Rats aged 6 hours to 14 days were exposed to white fluorescent light (*2*) delivering a spectral irradiance of 5 μW

Table 1. Change in serum calcium concentration after 3 hours of phototherapy. The results are expressed as means ± standard error. N.S., not significant.

Group	Age	Serum calcium (mg/dl)				Change in calcium (mg/dl)
		Shade	*N*	Phototherapy	*N*	
Control	6 hours	7.0 ± 0.13	4	6.2 ± 0.05	5	−0.8*
Control	18 to 24 hours	7.9 ± 0.11	13	7.1 ± 0.13	14	−0.8*
Control	1.5 to 2.5 days	8.6 ± 0.06	15	7.9 ± 0.06	19	−0.7*
Control	4 to 14 days	9.2 ± 0.12	29	8.3 ± 0.07	29	−0.9*
Gunn, icteric	2.5 days	7.5 ± 0.15	8	6.6 ± 0.24	8	−0.9*
Gunn, nonicteric	2.5 days	7.5 ± 0.13	8	6.6 ± 0.14	8	−0.9*
Jacket	18 to 24 hours	7.9 ± 0.18	4	7.1 ± 0.18	4	−0.8*
Jacket	1.5 days	9.4 ± 0.04	5	8.7 ± 0.10	6	−0.7*
Blindfold	1.5 days	8.7 ± 0.05	10	7.9 ± 0.08	9	−0.8*
Blindfold	4 days	9.5 ± 0.09	6	9.0 ± 0.11	6	−0.5*
Enucleated	1 to 4 days	8.9 ± 0.16	12	8.4 ± 0.13	13	−0.5†
Hood	1.5 days	9.4 ± 0.04	5	9.4 ± 0.07	12	0.0
Cap	1 to 2.5 days	8.3 ± 0.11	13	8.2 ± 0.09	13	0.0
Cap	4.5 days	9.0 ± 0.10	14	8.9 ± 0.10	13	0.0
Melatonin	3 to 4 days	8.7 ± 0.06	13	8.7 ± 0.07	24	0.0
Vehicle	3 to 4 days	8.8 ± 0.10	12	8.0 ± 0.09	22	−0.8*
Metyrapone	3 to 10 days	8.8 ± 0.17	11	8.6 ± 0.12	11	−0.2(N.S.)
Vehicle	3 to 10 days	8.4 ± 0.10	6	7.3 ± 0.08	7	−1.1
Blue light	4 to 5 days	8.8 ± 0.07	20	8.7 ± 0.07	17	−0.1(N.S.)

*$P < .01$. †$P < .05$ (Student's *t*-test).

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per square centimeter per nanometer at 425 to 475 nm (5) as determined by an Air Shields PR III phototherapy radiometer. In some groups, as noted below, certain areas of the body were shielded by application of black silk or India ink. Bilateral optic enucleation was performed in 28 rats aged 1 to 4 days. Of these, 15 received phototherapy and 13 served as shaded controls. Rats were separated from their mothers and confined in shallow plastic trays during light exposure; littermate controls were kept in identical trays under the light source but shaded by several thicknesses of opaque paper. To assess the role of hyperbilirubinemia, Gunn rats 3 days old with and without visible icterus were also used. Homozygotes of this strain have jaundice because they are deficient in glucuronyl transferase; heterozygotes have hyperbilirubinemia without visible jaundice. Blood was obtained by decapitation. Calcium was measured by EDTA titration (6) and bilirubin by spectrophotometry.

Since the size of the animals (7 g at 24 hours; 10 g at 4 days) precluded serial blood sampling, it was necessary to evaluate the effect of light by comparing the serum calcium concentration of rats exposed to light with that of littermates separated from their mothers but kept shaded. The effect of maternal deprivation was assessed in three litters of 18 to 24 hour old rats. Half of each litter was left with the mother; half was kept for 3 hours in shaded plastic trays under the light source. Serum calcium in the 12 separated rats was 7.9 ± 0.10 mg/dl (± standard error); in 11 littermates left with their mothers it was also 7.9 ± 0.10 mg/dl. We concluded that separation and tray confinement had no effect on serum calcium, and that differences between littermates kept in shaded and illuminated trays could be attributed to light.

Serum calcium in shaded rats increased with age from 7.0 mg/dl at 6 hours of age to 9.2 mg/dl at 4 to 14 days of age (Table 1). During phototherapy serum calcium decreased; the change (−0.8 mg/dl) was independent of the shaded control value in all age groups studied (Table 1). The decrease noted during light exposure was present at 1 hour and persisted unchanged for 6 hours; the average time of exposure for the rats listed in Table 1 was 3 hours.

Gunn rats, whether jaundiced or not, showed the same degree of hypocalcemia as normal rats (Table 1). Serum bilirubin decreased from 7.3 ± 0.24 mg/dl to 5.7 ± 0.17 mg/dl in jaundiced Gunn rats and from 3.1 ± 0.13 mg/dl to 2.4 ± 0.07 mg/dl in nonjaundiced littermates. In normal rats bilirubin before and after phototherapy was less than 0.5 mg/dl; available methods were not sufficiently precise to define a change at this level. Thus, decreases in bilirubin ranging from less than 0.5 to 1.6 mg/dl in 3 hours showed no correlation with simultaneous changes in calcium.

Table 2. Change in serum calcium concentration 1.5 hours after an injection of cortisol (0.5 nmole/g) and estradiol (1.1 nmole/g) with and without melatonin (4 nmole/g).

Treatment	N	Calcium (mg/dl)
Vehicle	19	8.8 ± 0.06
Melatonin	7	8.7 ± 0.19
Cortisol	10	8.0 ± 0.09*
Cortisol plus melatonin	15	8.9 ± 0.07
Estradiol	6	8.1 ± 0.08*
Estradiol plus melatonin	6	8.9 ± 0.14

*$P < .01$, compared to vehicle-injected controls (Student's t-test).

Shielding the trunk and limbs (jacket, Table 1), covering the eyes and forehead with opaque tape (blindfold, Table 1), or optic enucleation (enucleation, Table 1) did not prevent the hypocalcemic response to light. When the entire head was covered (hood, Table 1) or when the occiput was shielded by tape or India ink (cap, Table 1) serum calcium did not change during phototherapy. Results in the cap group suggested the possibility of pineal involvement.

Melatonin synthesis is inhibited by light, and serum melatonin concentrations fall precipitously during light exposure. In adult animals this effect is mediated by impulses originating in the retina and transmitted to the pineal via the accessory optic tract and superior sympathetic ganglion (7). In newborn rats, however, direct transcranial illumination inhibits melatonin synthesis even after optic enucleation (8). The effect of exogenous melatonin during phototherapy was therefore tested. Melatonin was dissolved in 4 percent ethanol (by volume) in normal saline and injected intraperitoneally immediately before light exposure. The dose was 1 μg (4 nmole) per gram and the injection volume 0.01 ml/g; this dose and route produce measurable effects within 20 minutes as evidenced by changes in brain serotonin content (9). Rats so treated showed no change in calcium on light exposure. The vehicle alone had no protective effect (melatonin, Table 1). Neither melatonin nor vehicle affected calcium in shaded rats.

Although the physiologic functions of melatonin are not well defined, there is evidence that it inhibits target organ responses to gonadal and adrenal steroids (10). In the rat, estradiol or cortisol administration at levels within the physiologic range induces acute hypocalcemia by increasing bone calcium uptake. This effect is greater in newborn animals than in intact adults, since the newborn period is characterized by functional hypoparathyroidism; parathyroidectomized adults show the same response as intact newborns, and exogenous parathyroid-hormone prevents the hypocalcemic response at all ages (11). We postulated that melatonin might influence calcium homeostasis by blocking the effect of steroids on bone calcium uptake.

We measured serum calcium in 3-day-old rats 1.5 hours after intraperitoneal injection of estradiol (1.1 nmole/g) or cortisol (0.5 nmole/g) with and without melatonin (4 nmole/g). These doses of estradiol and cortisol were those which we had previously found to produce in newborn rats plasma estradiol concentrations corresponding to concentrations of total estrogenic steroid in blood from the umbilical cords of human infants, and concentrations of cortisol corresponding to those of stressed newborn human infants (11). The vehicle was 10 percent (by volume) ethanol in normal saline (0.01 ml/g). Melatonin prevented the decrease in calcium after steroid injection (Table 2).

Since the hypocalcemic response to phototherapy was not realted to sex and persisted to age 14 days, it seemed unlikely that estradiol, of either placental or ovarian origin, was involved. We therefore studied the effect of inhibiting corticosterone synthesis on serum calcium during phototherapy. Metyrapone (25 μg/g, intraperitoneally in 0.01 ml of 10 percent ethanol, by volume, in normal saline) was given just prior to light exposure; at this dose level, metyrapone inhibits the 11-β hydroxylation essential to biosynthesis of cortisol and corticosterone from 11-desoxycortisol and desoxycorticosterone (12). Serum calcium did not decrease in the rats so treated (metyrapone, Table 1).

Rats exposed to blue light (2) delivering 12 μW per square centimeter per nanometer at 425 to 475 nm showed no change in calcium (Table 1). Transcranial penetration of blue and white light was compared by covering the sensor of a photographic light meter (Gossen Luna-Pro) with a disk comprising scalp, calvarium, and dura freshly excised from 5- to 11-day-old rats. The proportion of incident light transmitted was 17 percent

for white light and 9 percent for blue light at 5 days and 4 percent for white and 2 percent for blue light at 11 days. These findings correspond to a previous report (*13*) of light transmission through the crania of human infants, and may explain why blue light does not affect calcium.

Our results indicate that phototherapy-induced hypocalcemia is not related to bilirubin metabolism and can be prevented by occipital shielding, exogenous melatonin, or inhibition of corticosterone synthesis. Melatonin blocks the hypocalcemic effects of exogenous corticosteriod. We postulate that inhibition of melatonin synthesis results from transcranial illumination of the pineal, and that hypocalcemia ensues when bone calcium uptake is increased by the unopposed action of endogenous steroid.

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Increased Pyrophosphate in Fibroblasts and Lymphoblasts from Patients with Hereditary Diffuse Articular Chondrocalcinosis

Abstract. *The metabolic and genetic factors leading to deposition of calcium pyrophosphate crystals in cartilage of patients with chondrocalcinosis are not well understood. Analysis of cultured fibroblasts and lymphoblasts from 12 affected members of a large kindred showed a mean concentration of intracellular inorganic pyrophosphate two times greater than that in cells from unaffected family members or normal, unrelated volunteers. Increased intracellular pyrophosphate may, therefore, be a biochemical marker for the heterozygous expression of the chondrocalcinosis gene.*

In chondrocalcinosis, crystals of calcium pyrophosphate dihydrate are deposited in cartilage, leading to severe arthritis. The disease is found at autopsy in about 5 percent of the population. Chondrocalcinosis has been described in families in Czechoslovakia (*1*), Holland (*2*), Chile (*3*), France (*4*), Spain (*5*), and the United States (*6*). A dominant pattern of inheritance has been found in some families (*2, 4, 6*); in others the pattern of inheritance is less clear (*1, 3, 5*).

An increased concentration of inorganic pyrophosphate was previously reported in synovial fluid (*7–11*) in articular cartilage and in cultured chondrocytes and fibroblasts (*12*) from an affected patient, but not in plasma or urine (*7–13*). We now report an increased concentration of pyrophosphate in cultured fibroblasts and lymphoblasts from affected members of a large family with hereditary diffuse articular chondrocalcinosis (*4*).

Cells from three groups of subjects were cultured. The experimental group comprised 12 family members (seven females and five males 35 to 74 years of age), eleven of whom had had diffuse articular chondrocalcinosis for at least 10 years as confirmed clinically and radiologically. For one control group we selected unaffected siblings or close relatives of both sexes and 45 to 63 years of age. Unaffected, unrelated volunteers were used as a second control group.

As determined by the coupled enzymatic procedure (*14*), lymphoblasts and fibroblasts from affected family members had a substantially higher mean concentration of intracellular inorganic pyrophosphate than did cells from the control

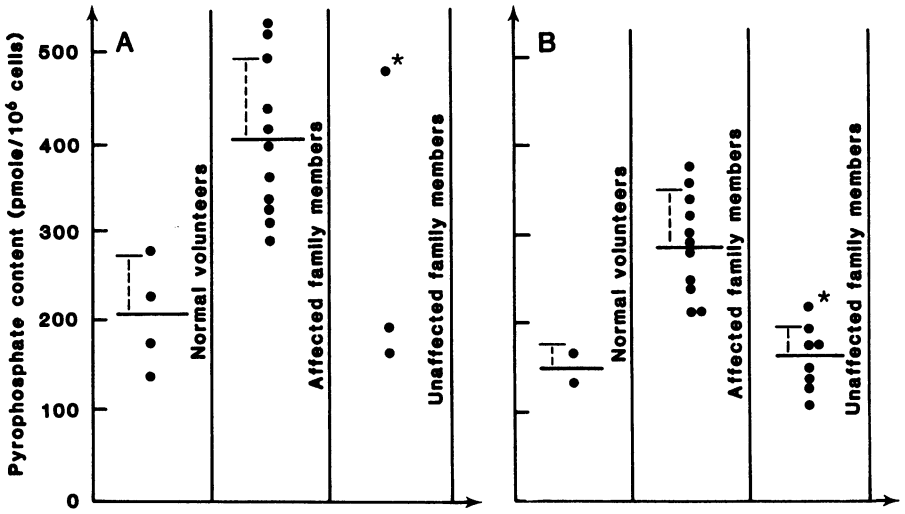

Fig. 1. Pyrophosphate content of fibroblasts (A) and Epstein-Barr–transformed lymphoblasts (B) from 12 affected family members, unaffected family members, and unrelated, unaffected volunteers. The mean concentration of intracellular inorganic pyrophosphate is 65 to 80 percent higher in affected family members than in the control groups. Fibroblasts were cultured in Eagle's minimal essential medium with 10 percent fetal calf serum, penicillin (50 U/ml), and streptomycin (50 μg/ml); the antibiotics were omitted after the first passage. The cells were then harvested and analyzed for pyrophosphate (*12*). Permanent lymphoblast lines were established by separating lymphocytes from peripheral blood by gradient density centrifugation in Ficoll Hypaque, adding Epstein-Barr virus, and culturing the cells in RPMI 1640 medium with 20 percent fetal calf serum (*17*). Asterisks indicate the subject who initially was classified as unaffected, but whose high pyrophosphate values prompted a clinical reexamination that produced x-ray evidence of chondrocalcinosis. The long horizontal lines denote means and the short horizontal lines show the magnitude of the standard deviations.

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