perconductivity theory (*8*). On the one hand, the attractive pair potential in the elements corresponds to the usual positive λ (*9*), and the correlation between the maximum T_s and softening (hard) modes is easily explained by the McMillan form (*8, 9*)

$$\lambda = C/\langle\omega^2\rangle \cdot M$$

On the other hand, the repulsive effect of a soft mode in the compounds is in agreement with Allen's investigation of this problem (*10*). However, since we are interested in the disappearance of superconductivity, the conditions of interest here are $T_s \sim \omega_q \ll \omega_0$, for which Allen's λ_a may become slightly negative. This conclusion is also supported by the more general analysis of Bergmann and Rainer (*11*). Indeed, for $T_s \ll \omega$ the change of T_s due to a spectral variation becomes inversely proportional to ω, $\delta T_s/\delta\alpha^2F(\omega) \propto \omega^{-1}$, which shows that, in the case $T_s \to 0$, the low-frequency part of $\alpha^2F(\omega)$ determines T_s. And since the Coulomb pseudo-potential μ^* becomes relatively more important, a change of sign of $\delta T_s/\delta\alpha^2F(\omega)$ cannot be excluded either.

The last point of our more elaborate analysis concerns the possibility of an electron-phonon induced Stoner instability. If $I_{(\mathrm{el-ph})}\chi^0(p, \omega; k, \nu)$ designates the iteration step in the electron-hole t-matrix (*7, 12*) due to phonon exchange, the contribution to the Stoner factor, averaged over the Fermi surface S_F, is given by

$$I_{(\mathrm{el-ph})}N(0) \equiv \langle I_{(\mathrm{el-ph})}\chi^0(p, p; 0, 0)\rangle_{S_F} = 2\int d\omega\alpha^2F(\omega) \times \left[\frac{1}{\omega} - \frac{1}{E_B - \omega} - \frac{\omega}{(\omega - i\delta)^2}\right]$$

Neglecting the bandwidth ($E_B \to \infty$), assuming a dominant soft and overdamped phonon, so that the imaginary part, δ, of the phonon self-energy exceeds the renormalized frequency ω, then $I_{(\mathrm{el-ph})}N(0) \cong \lambda$. Assuming further that the Coulomb part of the Stoner factor is just too weak to produce the instability we obtain the estimate

$$I_{(\mathrm{el-ph})}N(0) \gtrsim I_{\mathrm{total}}N(0) - 1 \cong 0.5 \times 10^{-4}$$

which is based on the values $T_m \cong 35$ K and $\epsilon_F \cong 0.2$ eV for $ZrZn_2$ (*13*). This shows that λ is probably quite small, of the order of 10^{-4}.

With regard to soft phonons it should be remembered that the most effective contribution to the phonon density of states $F(\omega)$ comes from a quasi one-dimensional soft mode, that is, one with a strong axial anisotrophy, since in this case $F(\omega) \propto (\omega - \omega_{q_0})^{-1/2}$, q_0 being the location of the soft mode in the Brillouin zone. In $ZrZn_2$ such highly directional soft modes can be qualitatively understood from the fact that the closed-shell configuration is $Zr^{4+}Zn^{2+}Zn^{6-}$ which points to a high degree of ionicity and to strong dielectric renormalization of the phonon frequencies (*8*). It is also in agreement with a narrow *d*-band (*13*) and with a strongly localized spin density (*14*).

A general survey of all *C* 15 compounds of the form ZrM_2 shows immediately why $ZrZn_2$ is such an exceptional case. All metals M, with the exception of Zn, are transition elements, and the resulting compounds are invariably superconducting. The effect of Zn in $ZrZn_2$, which has predominantly *s*− (instead of *d*−) orbitals, is to shield the *d*-electrons of Zr and hence leads to a lattice softening. A confirmation hereof can even be seen directly in its mechanical softness and low melting point. In the elements no such shielding exists so that, because of the stiffness of the *d*-electron binding, the corresponding phonon is a hard mode. This again is reflected by mechanical hardness and high melting temperatures.

The subtlety of our *p*-state pairing hypothesis is readily shown by the following two additional experimental facts. Replacing Zn_2 by CuAl a *C* 15 structure is again obtained. However, the softening of the Zn_2 and therefore the ferromagnetism are lost (*15*). Finally, gradual substitution of Zn by Fe destroys the ferromagnetism at less than 10 atomic percent Fe (*4, 15*) whereas, as is well-known, $ZrFe_2$ which has again the *C* 15 structure is ferromagnetic with a Curie point far above room temperature.

As was stated at the outset, the only other compound known to be an itinerant ferromagnet is Sc_3In. Since similar arguments are likely to apply again, the rarity of this type of compound becomes evident. Will there ever be a third itinerant ferromagnet? Isostructural and isoelectronic with $ZrZn_2$ is only $TiBe_2$. Thus far we only know that it is not superconducting above 1 K.

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References and Notes

1. J. K. Hulm and R. D. Blaugher, *Phys. Rev.* **123**, 1569 (1961); E. Bucher, F. Heiniger, J. Müller, in *Proceedings of the 9th International Conference on Low Temperature Physics* (Plenum, New York, 1965), LT9, pp. 482–486; B. T. Matthias, unpublished data.
2. S. Ogawa, *Phys. Lett. A*, **25**, 516 (1967).
3. H. J. Blythe and J. Crangle, *Phil. Mag.* **18**, 1143 (1968).
4. S. Ogawa, *J. Phys. Soc. Jpn.* **25**, 109 (1968).
5. J. Bardeen, L N. Cooper, J. R. Schrieffer, *Phys. Rev.* **108**, 1175 (1957).
6. P. W. Anderson and P. Morel, *ibid.* **123**, 1911 (1961).
7. S. Doniach and S. Engelsberg, *Phys. Rev. Lett.* **17**, 750 (1966).
8. D. J. Scalapino, in *Superconductivity*, R. D. Parks, Ed. (Dekker, New York, 1969), vol. I, p. 449.
9. W. L. McMillan, *Phys. Rev.* **167**, 331 (1968).
10. P. B. Allen, *Solid State Commun.* **12**, 379 (1973).
11. G. Bergmann and D. Rainer, *Z. Phys.* **263**, 59 (1973). We thank E. Müller-Hartmann for having brought this paper to our attention.
12. N. F. Berk and J. R. Schrieffer, *Phys. Rev. Lett.* **17**, 433 (1966).
13. D. D. Koelling, D. L. Johnson, S. Kirkpatrick, F. M. Mueller, *Solid State Commun.* **9**, 2039 (1971).
14. S. J. Pickart, H. A. Alperin, G. Shirane, R. Nathans, in *Proceedings of the International Conference Magentism, Nottingham* (1964), p. 223.
15. B. T. Matthias, unpublished data.
16. Research at La Jolla was sponsored by NSF/DMR77-08469.
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Jaundice Phototherapy: Micro Flow-Cell Photometry Reveals Rapid Biliary Response of Gunn Rats to Light

Abstract. *Hepatic pigment clearance in rats can be followed continuously with photometric detectors designed for high-pressure liquid chromatography. This method showed that light has a fast effect on bilirubin metabolism in homozygous Gunn rats, even at low doses and intensities. This is consistent with geometric isomerization of bilirubin IXα as a primary step in phototherapy.*

Phototherapy is an established procedure in neonatal medicine (*1*). It is used prophylactically on babies with neonatal jaundice to prevent bilirubin encephalopathy, which is caused by deposition of bilirubin IXα in the brain. Ephemeral jaundice is common during the early neonatal period because the liver is functionally immature and often unable to clear bilirubin IXα from the circulation in the normal way (that is, by hepatic uptake, glucuronidation, and excretion). In phototherapy, endangered infants with high or rapidly rising plasma levels of bilirubin are irradiated with blue or white lights to diminish circulating bilirubin IXα and reduce the risk of its diffusion into the brain. Usually, irradiation is

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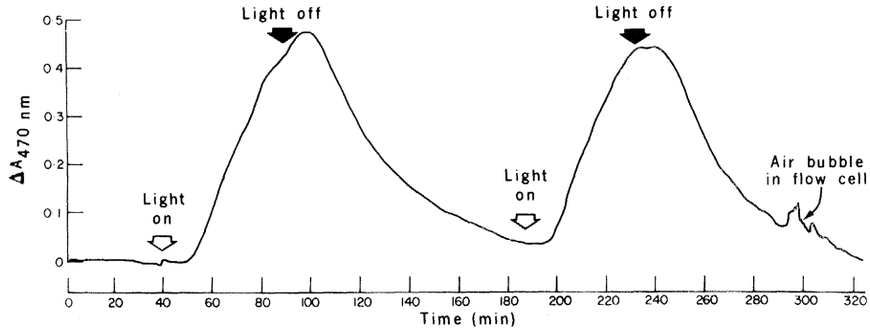

Fig. 1. Changes in absorbance (470 nm) of homozygous Gunn rat bile in response to two 45-minute periods of irradiation. Absorbance was measured with an 8-μl capacity, 2.0-mm path-length flow-cell, and the rat was infused intravenously throughout at 3 ml per hour with an aqueous solution containing sodium cholate ($3.0 \times 10^{-2} M$), egg lecithin (1.5 g per liter), cholesterol ($1.6 \times 10^{-4} M$), and taurine ($3.0 \times 10^{-2} M$). The light fixture contained eight Westinghouse 20-W Special Blue F20T12/BB lamps. These emit one main emission band in the visible region between 380 and 540 nm with maximum emission at ~ 450 nm. At the rat's back the spectral irradiance, measured at the wavelength of peak emission, was 23 μW cm^{-2} nm^{-1}, and total irradiance from 400 to 520 nm was 0.95 mW cm^{-2}.

continued for many hours until plasma bilirubin concentrations are acceptably low or hepatic excretion is fully functional. The complete mechanism of phototherapy is not known. But it is known that the treatment causes excretion of intact unglucuronidated bilirubin IXα in the bile and formation of obscure bilirubin derivatives, possibly photooxidation products, that are excreted in bile and urine (*1, 2*). These effects are manifested clinically by slow blanching of the skin (*3*) and a slow decline in the concentration of bilirubin IXα in the blood. Since these signs do not become apparent for hours, jaundice phototherapy is considered generally to involve rather slow photobiological processes. Using a new sensitive method for measuring hepatic pigment excretion continuously in the rat, we have found the contrary. Though the gross effects of phototherapy may be slow, the physiologic response to light is fast.

Homozygous male Gunn rats weighing 300 to 450 g were used. These animals are genetically devoid of bilirubin glucuronyl transferase and are unable to conjugate and excrete bilirubin IXα like other mammals. Consequently, they have a lifelong unconjugated hyperbilirubinemia and provide a useful, unique animal model for studying phototherapy of neonatal jaundice (*2*). When homozygous Gunn rats are illuminated, the optical density of the bile remains constant from ~ 550 to 800 nm but increases at shorter wavelengths (~ 360 to 520 nm) (*2*) with the largest increase at ~ 420 to 470 nm. We have exploited this effect to follow the response of these rats to irradiation. To do this the absorbance of bile was measured continuously at 470 nm (A_{470}) by surgically connecting the common bile duct with PE-90 tubing (10 cm) directly to a micro flow-cell housed in an electronic absorption detector (*4*). The flow-cell and photometric detector were the type used in high-pressure liquid chromatography detector systems. This equipment is suitable for measuring absorbance changes in pigmented neat bile since the cell path-length is short (2 mm) and high absorbancies can be accommodated. In addition, the pressure drop across the flow-cell is negligible so that there is little impedance to bile flow, and the cell capacity and total dead space are small (8 and 143 μl, respectively).

The following general procedure was used. The rat's back was depilated and cannulas were inserted into the common bile duct and a femoral vein (*2*). The animal was then placed in a restraining cage 15 to 19 cm beneath a horizontal fixture containing blue fluorescent lights. The restraining cage was constructed so that most of the dorsal skin surface could be exposed to the light while keeping the nape, head, and eyes shielded. The bile cannula was connected to the flow-cell situated beneath the rat, and infusion of a synthetic "bile" replacement mixture was started through the femoral cannula. The infusion stabilized bile flow and prevented adsorption of bile pigments on internal surfaces of the apparatus. The animal was kept in the "dark" under an infrared heating lamp for 1 to 2 hours to allow bile flow rate and A_{470} to become constant. Then the light was switched on and changes in A_{470} were recorded continuously. Throughout each experiment bile flow rates, which were approximately 2 ml per hour, remained constant to within 0.1 ml per hour.

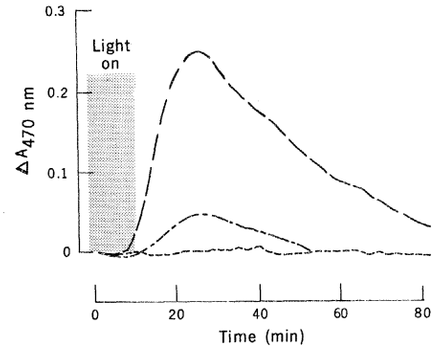

Fig. 2. Changes in absorbance (470 nm) of homozygous Gunn rat bile in response to two 10-minute pulses of light with different intensities. The lower tracing was obtained with the rat in the dark throughout, the top curve is the response to irradiation for 10 minutes with the light at full intensity, and the middle curve is the response to a 10-minute irradiation with the light dimmed by reducing the voltage supply. For the top curve the maximum spectral irradiance at the rat's back was 28 μW cm^{-2} nm^{-1} and total irradiance (400 to 520 nm) was 1.25 mW cm^{-2}; for the middle curve the corresponding values were 7 μW cm^{-2} nm^{-1} and 0.30 mW cm^{-2}. The three curves were determined with the same animal in the sequence bottom, top, middle. For other experimental details see Fig. 1.

Figure 1 shows a typical response to two successive brief periods of illumination, in this instance 45 minutes each. During the first dark period A_{470} of the bile, arbitrarily set at zero, remained relatively constant for 40 minutes. Ten minutes after switching on the light A_{470} began to increase sharply. About 5 minutes of this 10-minute lag was the time required for bile to flow from the liver to the optical detector. Therefore excretion of pigment into bile actually began within 5 minutes of starting the irradiation. Conversely, the absorbance began to decrease 10 minutes after the light was switched off, and this decrease continued until the basal absorbance was reached again. On reirradiation the same pattern was observed. The second time enhanced pigment excretion, after correction for bile transit time, was detectable within 3 minutes of switching on the light. During the experiment the concentration of bilirubin in the serum was not measured. But it is unlikely that it would have shown a significant change, because in control experiments with the same light source more than 5 hours of continuous irradiation was required to produce a significant decrease, in broad agreement with previous observations (*2, 5*).

The sharp, rapid rise in A_{470} was readily detectable with much smaller doses of light. Figure 2 shows the response of a jaundiced Gunn rat to two 10-minute pulses of light, one with the light at normal intensity, the other with the irradiance reduced fourfold. The curves are qualitatively similar to those of Fig. 1, and for the higher irradiance curve, en-

hanced pigment excretion again was detectable 5 minutes after illumination began.

These results show that the physiologic response of jaundiced rats to blue light is mediated via the skin surface without involving pineal or visual photoreceptors. As in vision or phytochrome-mediated processes in plants, the photobiologic response is fast even at low light intensities. Pronounced changes in bile composition are observed in minutes, in contrast to the hours of phototherapy required to produce significant changes in plasma bilirubin concentration. Obviously, the plasma bilirubin concentration is not a sensitive or direct measure of the photobiologic response, and its value in studying the mechanism and kinetics of phototherapy is limited.

The mechanism for the enhanced excretion of unconjugated bilirubin during phototherapy is unknown. A current hypothesis cites geometric isomerization (*6*). Bilirubin IXα, which has two *Z*-configuration bridge double bonds (*7*) and requires conjugation for excretion, is thought to be converted by absorption of light to *E-Z* or *E-E* isomers that can be excreted without conjugation. Our observations are compatible with this theory and consistent with immediate formation of a compound in the skin that migrates to the blood, is taken up by the liver, and is rapidly excreted in bile. Although increased pigment excretion is detectable very soon after illumination begins, the response is rather slow compared to the excretion of exogenous anions such as bromosulfophthalein or rose bengal, which appear in bile less than 1 minute after an intravenous pulse injection (*8*). Quite likely, pigment excretion in phototherapy is delayed somewhat because the putative bilirubin isomers or other pigments that are generated photochemically have to pass from extravascular tissues to plasma before they can be cleared and excreted.

Additionally, these studies show that high-pressure liquid chromatography flow-cell detectors, which are readily available, can be used to monitor pigment concentrations in rat bile continuously and directly. This sensitive technique has several advantages over the usual batch-sampling methods and is valuable for studying the hepatic excretion and metabolism of endogenous and exogenous pigments (*8*).

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References and Notes

1. A. K. Brown and J. Showacre, *Phototherapy for Neonatal Hyperbilirubinemia* [U.S. Department of Health, Education, and Welfare Publication No. (NIH) 76-1075, 1977]; R. E. Behrman *et al.*, *J. Pediatr.* **84**, 135 (1974); L. M. Gartner and K.-S. Lee, in *Neonatal-Perinatal Medicine*, R. E. Behrman, Ed. (Mosby, St. Louis, 1977), pp. 394–415.
2. J. D. Ostrow, *J. Clin. Invest.* **50**, 707 (1971).
3. T. P. Vogl, *J. Pediatr.* **85**, 707 (1974).
4. The detector used was a model 153 fixed-wavelength, dual-beam detector made by Altex Scientific Inc., Berkeley, Calif., and fitted with a 470-nm wavelength filter. Further details of this procedure will be published elsewhere (L. M. Ramonas and A. F. McDonagh, in preparation).
5. J. R. Hewitt, R. M. Klein, J. F. Lucey, *Biol. Neonate* **21**, 112 (1972).
6. D. A. Lightner, *Photochem. Photobiol.* **26**, 427 (1977); A. O. Pedersen, F. Schønheyder, R. Brodersen, *Eur. J. Biochem.* **72**, 213 (1977).
7. R. Bonnett, J. E. Davies, M. B. Hursthouse, *Nature (London)* **262**, 326 (1976).
8. L. M. Ramonas and A. F. McDonagh, in preparation.
9. This work was supported by grants (NIAMDD) P50 AM-18520 and AM-18220 from the National Institutes of Health. We thank Leon H. Johnson and Carl Shackleford for advice and assistance.

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Time-Resolved Resonance Raman Spectroscopy of Hemoglobin Derivatives: Heme Structure Changes in 7 Nanoseconds

Abstract. *Resonance Raman spectra of oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and the corresponding myoglobin derivatives have been obtained with 7-nanosecond laser pulses at 531.8 nanometers. The results suggest that no transient constraint of the heme group by the globin structure occurs on this time scale, and thus establish a temporal sequence for the early events that may participate in the stereochemical trigger mechanism of hemoglobin cooperativity.*

We have recorded the resonance Raman spectra of oxyhemoglobin (O_2Hb), deoxyhemoglobin (deoxy Hb), carboxyhemoglobin (COHb), and the corresponding myoglobin derivatives, using 7-nsec excitation pulses at the second harmonic wavelength (531.8 nm) of an Nd:YAG laser. In the case of COHb, essentially complete photodissociation of CO from the heme iron occurs in the first few hundred picoseconds during which the laser pulse impinges on the sample. Therefore, the observed resonance Raman scattering is predominantly due to photodissociated COHb. Inasmuch as the protein structural relaxations that follow photodissociation of COHb are thought to occur with half-lives of 40 nsec or longer (*1*), the "COHb" spectra that we observe are actually spectra of hemoglobin in which the heme group is deoxy, but the protein remains predominantly in its ligated (oxy) tertiary and quaternary structures. Nevertheless, the time-resolved resonance Raman spectra of photodissociated COHb appear insignificantly different from those of deoxy Hb. The implications of this result for the dynamics and statics of hemoglobin cooperativity are discussed.

The apparatus employed to record the time-resolved resonance Raman spectra will be described in detail elsewhere (*2*). Resonance Raman scattering was excited by repetitive (5 to 20 Hz) pulses from a *Q*-switched frequency-doubled Nd:YAG oscillator (Quanta-Ray DCR-1). This laser is capable of delivering 75 mJ pulses at 531.8 nm; the energy per pulse used in this study was varied between 3 and 27 mJ. The measured pulse width at 531.8 nm was 7 nsec (full width at half-maximum). Raman-scattered light from the sample was collected at $f/1$ using essentially conventional illuminator optics, and focused at $f/7$ into a vidicon spectrograph consisting of a Spex 1870 0.5-m spectrograph with a Princeton Applied Research 1205I detector head in the image plane. This arrangement allowed simultaneous detection of a 345 cm^{-1} segment of the Raman spectrum with peak positions accurate to within 2 cm^{-1}. Spectra were processed by the Princeton Applied Research optical multichannel analyzer console and recorded on an X-Y plotter. Hemoglobin was isolated from human whole blood by a modification of the method of Drabkin (*3*). Resonance Raman spectra were obtained at 25°C in aqueous solution, *p*H 7.0, at a total heme concentration of 0.5 to 1.0 m*M*. Oxyhemoglobin was observed in air-saturated solutions and deoxyhemoglobin was formed by reduction with excess sodium dithionite. Carboxyhemoglobin was formed from the deoxy Hb solution by addition of CO and was observed under 1 atm of CO. Myoglobin was obtained from Sigma (type I, from equine skeletal muscle) and observed under the same conditions as hemoglobin.

The generally accepted model for hemoglobin cooperativity proposes that the "stereochemical trigger" for the change from the ligated (oxy tertiary and R quaternary) to the unligated (deoxy tertiary and T quaternary) protein conformation is the spin-state change of the iron atom on deligation at the axial site *trans* to the proximal histidine (*4, 5*). This spin-state change is accompanied (or followed) by

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