GA II cannot distinguish the parents from normal individuals although the method measures half-normal activities in GA I parents (see Table 7). An explanation for this discrepancy could be that GDH is the limiting factor in this assay as indicated by the intermediate values in the obligate GA I heterozygotes. Half-normal concentration of ETF or ETF-DH would then still be enough for full GDH activity. Another possibility is that the synthesis of the missing enzyme in GA II (*i.e.* ETF or ETF-DH) is regulated so the one gene that is functioning in GA II heterozygotes keeps working until a normal enzyme level is reached. This cannot occur in GA II homozygotes where both genes are defective.

Although the normal GDH activity measured with intact ETC as EA is a disadvantage in the diagnosis of GA II heterozygotes, it makes prenatal diagnosis of GA II homozygotes easier because of the high difference in enzyme activities between GA II heterozygotes and homozygotes.

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15. This research was supported by the Danish Medical Research Council.
16. The authors acknowledge the expert technical assistance of Mrs. Eva Korup, Mrs. Vibeke Winter, and Mrs. Mette Houman Møller.
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18. Received for publication August 6, 1983
19. Accepted for publication October 19, 1983

0031-3998/84/1807-0667$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 7, 1984
Printed in U.S.A.

Phototherapy for Neonatal Jaundice: *in Vitro* Comparison of Light Sources

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Summary

Phototherapy results in the conversion of bilirubin to more water-soluble isomers. Six clinically used phototherapy lamps which differ in their emission spectra have been compared in their ability to produce configurational and structural isomers of bilirubin *in vitro*. For all of the lamps, the initial rate of configurational isomerization was highly correlated ($r = 0.969$) with the intensity of irradiation falling within the bilirubin absorption band. The percentage of the total bilirubin converted to the configurational isomer at equilibrium was dependent upon the spectral distribution of the lamp, and was greatest (26.2 ± 1.3%) with the special blue lamp, which has a narrow spectral output centered at 445 nm. The rate of formation of the structural isomer, lumirubin, was generally dependent upon the intensity of irradiation within the bilirubin absorption band.

Abbreviation

HEPES, *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

Despite the widespread use of phototherapy for the prevention and treatment of neonatal jaundice, debate continues over the most effective light source (2, 6, 15–19). Thus, several phototherapy units which differ in spectral distribution and intensity have been recommended. These recommendations are based on empirical studies which compared the effect of different light sources on serum bilirubin concentration in jaundiced infants (15–19) or congenitally jaundiced Gunn rats (2). A limitation of such studies is that the measured effect of phototherapy, *i.e.* decline in serum bilirubin, is a secondary and delayed response (3, 10) (Ennever, Knox, Denne, and Speck, submitted). The primary effect of the treatment is rapid conversion of bilirubin to excretable photoproducts (8, 13, 14) (Ennever *et al.*, submitted). These include yellow compounds which are isomers of bilirubin (*i.e.* which have the same chemical formula) (11, 13). The decline in serum bilirubin requires transport of these photoisomers from their site of formation to the liver where they are excreted in the bile (14). Recent studies have shown that the rates of excretion of these photoproducts are different in the

human newborn infant than in the Gunn rat (14) (Ennever *et al.*, submitted). Therefore, data derived from the Gunn rat on the relative efficacy of various phototherapy lamps may not be directly applicable to the human neonate.

Previous studies have shown that, during phototherapy of jaundiced infants or irradiation of bilirubin-human albumin solutions *in vitro*, the most rapid photochemical reaction is the formation of a configurational isomer of bilirubin, designated as 4*Z*,15*E*-bilirubin (8, 12). When the reaction is carried out *in vitro*, an equilibrium is eventually established between the natural 4*Z*,15*Z* form and the 4*Z*,15*E* isomer (6, 12). An additional photochemical reaction, which occurs at a slower rate both *in vitro* and *in vivo*, is the formation of a structural isomer, designated lumirubin (11) (Ennever *et al.*, submitted). Recently, we have shown that both the rate of formation (4) and the equilibrium concentration (6) of the 4*Z*,15*E* configurational isomer produced by monochromatic irradiation *in vitro* is highly wavelength dependent. It is difficult to extrapolate these results to the broad spectrum lights commonly used in phototherapy. Therefore, we have selected several phototherapy lamps that differ in their spectral emission characteristics and have compared their ability to produce configurational and structural isomers of bilirubin *in vitro*. These data provide the background necessary for similar comparative studies in jaundiced infants.

MATERIALS AND METHODS

Bilirubin (Sigma Chemical Co., St Louis, MO), purified as previously described (9), was dissolved in 0.01 M NaOH and added to a solution of human serum albumin (fatty acid free, Sigma) in 0.15 M NaCl 0.05 M HEPES, pH 7.5. After addition of the alkaline bilirubin solution, the pH of the solution was restored to 7.4 by the addition of 0.01 M HCl. Final concentrations were 44 μM bilirubin and 88 μM albumin.

Five of the light sources were fluorescent lamps (20 watt): special blue (Westinghouse F20T12/BB), blue (Sylvania F20T12/B), daylight (Sylvania F20T12/DA), green (Sylvania F20T12/G), and Vita-Lite (DuroTest). Before use, the lamps were aged for 100 h to obtain a stable spectral output (7). The one additional light source was a Cavitron PT 1400 phototherapy unit containing a tungsten-halogen filament lamp (General Electric type ENH).

Fifteen-milliliter samples of the bilirubin-albumin solution were placed in 30-ml plastic culture flasks (Costar, Cambridge, MA), resulting in a 3.5 × 3.5 × 1.3 cm volume of liquid. Samples were irradiated normal to the square surface, positioned 50 cm from either a bank of eight fluorescent lamps in a standard phototherapy canopy or from a single tungsten-halogen lamp. During irradiation, the samples were magnetically stirred and thermostated to 20°C. Aliquots were removed at timed intervals and the formation of bilirubin photoproducts was followed by high pressure liquid chromatography on a 0.46 × 25 cm Zorbax-ODS column (Dupont Co., Wilmington DE) fitted with a precolumn. The eluant was 0.1 M di-*n*-octylamine acetate in methanol, pH 7.7 (12). Chromatography was performed on a Varian model 5060 liquid chromatograph equipped with a model UV-100 detector (Varian Associates, Walnut Creek, CA) set at 465 nm and a Hewlett-Packard (Avondale, PA) model 3390A reporting integrator. The integrated peak areas were not corrected for differences in the 465-nm extinction coefficient among the various bilirubin isomers.

Emission spectra of the lamps were recorded on Cary model 118 or 210 spectrophotometers (Varian Associates). Emission spectra were recorded in the single beam mode with the phototherapy lamps substituted for the spectrophotometer light source. Irradiance at 50 cm was measured normal to each lamp with an International Light model IL700 radiometer and a SEE 400 broad-band detector fitted with a filter (type TBLU, International Light) which transmitted light between 350 and 530 nm. The peak response of this detector-filter combination was at 468 nm with half-maximum points at 397 and 483 nm.

RESULTS AND DISCUSSION

When solutions of bilirubin bound to human serum albumin are exposed to light, the fastest reaction is configurational isomerization about the meso double bond between carbons 15 and 16 [configurational isomerization at the C-4–C-5 meso double bond does not occur when bilirubin is bound to human albumin (11)]. This reaction can be represented by:

$$4Z,15Z\text{-bilirubin} \xrightarrow{h\nu} 4Z,15E\text{-}bilirubin \qquad (1)$$

where $h\nu$ represents a photon of frequency ν. The results in Table 1 demonstrate that the *initial* rate of this reaction, expressed as the inverse of the time required to convert 10% of the native bilirubin to the 4*Z*,15*E* isomer ($1/T_{10}$), is dependent upon the intensity of irradiation falling within the absorption band of bilirubin ($I_{400\text{-}500}$). The correlation between these two parameters is 0.969.

The configurational isomerization reaction (Equation 1) is photochemically reversible (6, 12) and must compete with the corresponding reverse reaction (the 4*E*,15*E* isomer is not formed in detectable amounts):

$$4Z,15Z\text{-bilirubin} \underset{h\nu}{\overset{h\nu}{\rightleftarrows}} 4Z,15E\text{-}bilirubin \qquad (2)$$

Thus, in a closed system, an equilibrium is eventually established. Because the two isomeric forms have overlapping, but not identical, absorption spectra (6), the position of this equilibrium (Equation 2) will depend upon the spectral distribution of the light source being used. With monochromatic irradiation, the percentage of the total bilirubin present as the 4*Z*,15*E* isomer at equilibrium ranged from 16% with 490 nm light to greater than 40% with 390 nm light (6). The table gives the percentage of the total bilirubin present as the 4*Z*,15*E* isomer at equilibrium with the six phototherapy lamps. The greatest equilibrium concentration of 4*Z*,15*E* configurational isomer was achieved with the special blue lamp, which has a narrow spectral output centered at 445 nm (Fig. 1). Lower equilibrium concentrations were produced by the three fluorescent lamps with broad emission spectra [daylight, Vita-Lite, and blue (Fig. 1)]. A somewhat lower equilibrium concentration was produced by the tungsten-halogen lamp despite its rapid initial rate of isomerization. The least conversion of native bilirubin to the 4*Z*,15*E* isomer was achieved with the green lamp [recently used experimentally in the treatment of neonatal hyperbilirubinemia (18)], which has little emission falling within the absorption band of bilirubin (Fig. 1).

Table 1 shows the percentage of the total bilirubin present as the structural isomer lumirubin after 20 and 60 min of irradiation with the six phototherapy lamps. The greatest conversion of bilirubin to lumirubin was achieved by the two lamps (special blue and tungsten-halogen) with the greatest irradiance within the bilirubin absorption band. The green lamp, with the least emission in the 400 to 500 nm region, produced lumirubin at a rate comparable to that of the remaining three fluorescent lamps (daylight, Vita-Lite, and blue). This result is somewhat surprising. Some enhancement of the rate of lumirubin formation by the green lamp would be expected because this lamp forms the least 4*Z*,15*E* isomer. The 4*Z*,15*E* isomer is not a precursor of lumirubin (11) and its presence diminishes the rate of lumirubin formation both by reducing the concentration of native bilirubin and by acting as an internal filter. An additional enhancement of the rate of lumirubin formation by the green lamp would result if this photochemical reaction is favored by light at longer wavelengths. The action spectrum for conversion of bilirubin to lumirubin has not been measured and will be difficult to determine because of the competing and much more rapid formation of 4*Z*,15*E*-bilirubin. Recent studies indicate that lumirubin is formed directly from bilirubin and not from the 4*Z*,15*E* isomer (11). Therefore, the action spectrum for lumirubin formation *in vitro* is probably coincident with, or at least contained within, the absorption spectrum of bilirubin.

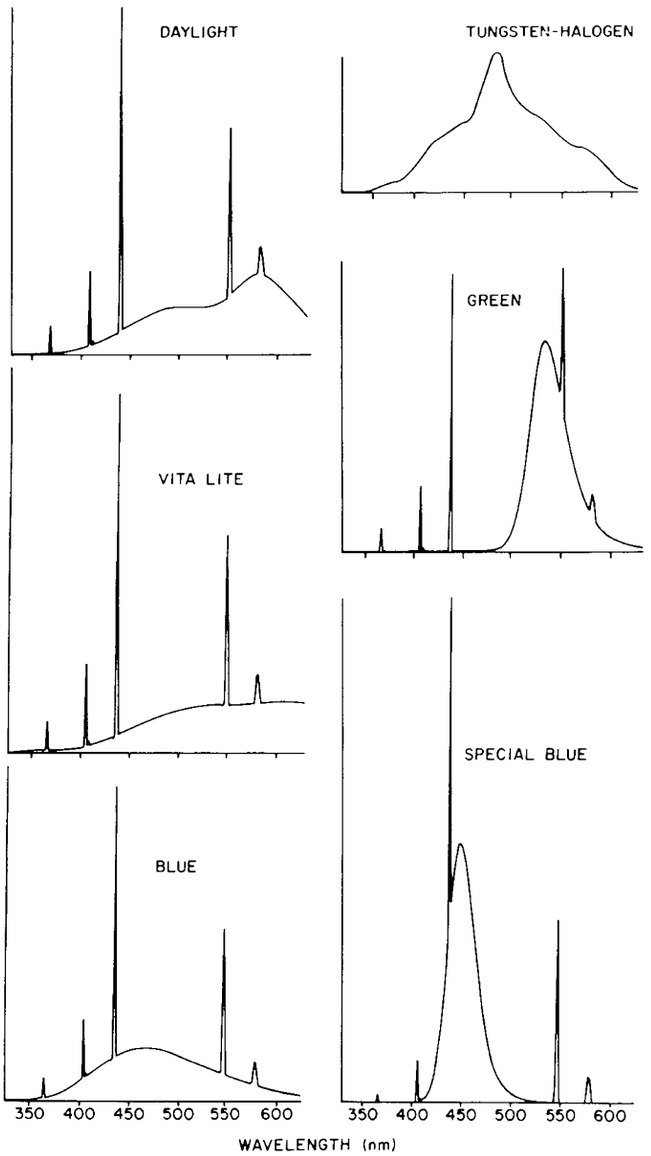

Fig. 1. Emission spectra of phototherapy lamps. The intensities are shown on a linear relative scale and were measured as described in "Materials and Methods." The spectra of the five fluorescent lamps (daylight, Vita-Lite, blue, special blue, and green) were measured under identical conditions on a Cary 118 spectrophotometer. The spectrum of the tungsten-halogen lamp was measured on a Cary 210 spectrophotometer.

This study provides the first comparison of the efficacy of clinically used phototherapy light sources in the biologically important configurational and structural isomerization reactions of bilirubin *in vitro*. These data cannot be extrapolated directly to *in vivo* phototherapy because of the influence of the optical properties of the skin (1), and because the relative contributions of configurational and structural isomerization to the therapeutic response to phototherapy are not known. The absorption of light by the skin will diminish the effective irradiance *in vivo* and thus diminish the initial rates of these reactions. In addition, light scattering preferentially attenuates the shorter wavelengths (1), and thereby alters the effective spectral distribution *in vivo* and the relative rates of lumirubin and 4*Z*,15*E* isomer formation. In particular, light sources such as the daylight lamp whose emission within the bilirubin absorption band contains a substantial contribution from shorter wavelengths would probably be less effective *in vivo* than suggested by our *in vitro* results.

Certain conclusions can be drawn about the *in vivo* efficacy of commonly used phototherapy lamps based upon our *in vitro* data. The broad spectrum fluorescent lamps (daylight, Vita-Lite, and blue) and the green lamp produce the lowest rates of configurational and structural isomerization, and therefore are probably least effective *in vivo*. The special blue and the tungsten-halogen lamps produce rapid rates of isomerization and are therefore probably the most effective of lamps currently used clinically.

A recently completed clinical study suggests that lumirubin formation and excretion may be an important pathway for bilirubin elimination during phototherapy (Ennever *et al.*, submitted). If lumirubin formation is important and if it is favored, as our *in vitro* data suggest, by longer wavelength light, then a more effective lamp for phototherapy would be one with a spectral output similar to that of the special blue with its emission band shifted or broadened on the longer wavelength side.

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Table 1. *Isomerization of bilirubin by different phototherapy light sources*

	Green	Vita-Lite	Daylight	Blue	Special blue	Tungsten-halogen
$I_{400-500}$ ($\times 10^4$ watts/cm^2)	1.4	3.5	5.1	6.6	13.4	18.9
Initial rate ($1/T_{10}$) (sec^{-1} $\times$ 100)	0.63†	1.06	1.39	2.1	3.5	3.7
	(0.05)	(0.02)	(0.04)	(0.1)	(0.3)	(0.2)
4*Z*,15*E* at equilibrium (%)	6.9	17.5	18.7	19.8	26.2	14.1
	(0.7)	(0.8)	(1.0)	(1.0)	(1.3)	(0.8)
Lumirubin after 20 min (%)	1.7	1.4	1.6	2.1	3.0	6.1
	(0.4)	(0.4)	(0.2)	(0.4)	(0.2)	(0.7)
Lumirubin after 60 min (%)	4.1	3.1	4.1	4.6	6.9	14.4
	(0.2)	(0.2)	(0.2)	(0.1)	(0.1)	(0.4)

* Values in parentheses are the standard deviation of three or more experiments.

† Calculated by extrapolation from initial rate of configurational isomerization because final percentage of 4*Z*,15*E* isomer was only 6.9.

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21. This research was supported by the United States Public Health Service through Grants CA-23692, AM-26307, and AM-11275, by Research Career Development Award IK0-0043 to W. T. S., and by a grant from the Board of Trustees of Rainbow Babies and Childrens Hospital.
22. Received for publication September 26, 1983.

0031-3998/84/1807-0670$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 7, 1984
Printed in U.S.A.

A New Form of Insulin Resistance with Growth Retardation, Fatty Liver, and Hypogonadotropic Hypogonadism

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Summary

A 17-year-old boy presented with growth retardation, marked hepatomegaly, and sexual infantilism. Elevated fasting serum insulin levels and a blunted hypoglycemic response to exogenous insulin (up to 0.35 unit/kg) demonstrated severe insulin resistance. Neither anti-insulin nor anti-insulin receptor antibodies were present. The molecular size of his circulating insulin and its binding to IM-9 lymphocytes was normal. Despite high circulating insulin values, both erythrocytes and cultured skin fibroblasts showed normal insulin binding capacity and affinity. Tissue responsiveness was examined by measuring the insulin-induced increase in 2-deoxyglucose uptake into fibroblasts. Although the basal glucose transport rate was slightly lower than that of controls, the insulin-induced increase was normal. However, the normal increase in thymidine incorporation in response to insulin was blunted, as were the thymidine incorporation responses to epidermal growth factor and fibroblast growth factor. These studies demonstrate the possible existence of a new form of post-insulin receptor defect as a cause of insulin resistance, but underscore the difficulty that exists in defining the exact nature of the defect in these disorders.

Abbreviations

IRI, immunoreactive insulin
G, gonads
PH, pubic hair
EGF, epidermal growth factor
hFGF, human fibroblast growth factor
Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
2-DG, 2-deoxyglucose

Insulin resistance, a clinical syndrome characterized by a blunted response to endogenous insulin, with or without a similar poor response to administered insulin, causes some degree of hyperglycemia as well as other more variable clinical features (2, 26). Its etiology may include production of an abnormal insulin molecule (6, 33), the presence of circulating antagonists to insulin such as anti-insulin or anti-insulin receptor antibodies and contra-insulin factors (5, 25, 36), abnormally rapid degradation of insulin (27), quantitative (32) and qualitative (18, 35) abnormalities of the insulin receptors (18), and poorly defined defects in post-receptor mechanisms (19, 22).

This report describes a 17-year-old boy with an unusual form of insulin resistance in whom extensive studies point to a post-receptor defect but have failed to define the exact nature of the abnormality.

CASE REPORT

A 17-year-old Caucasian boy was referred for evaluation of hepatomegaly and growth retardation. He was the product of an uneventful 37-week pregnancy with a birth weight of 2.2 kg. He showed no dysmorphic features other than bilateral cryptorchidism. There was no family history of consanguinity or diabetes. The midparental stature was 161.3 cm. His height and weight growth was below but parallel to the third percentile throughout childhood. Orchidopexy was carried out at 6 years of age; at age 8, his serum follicle-stimulating hormone, luteinizing hormone, and testosterone values were in the normal prepubertal range. At age 15, hepatomegaly was first noticed. Biopsy of the liver demonstrated severe fatty metamorphosis; there was appreciable glycogen content and moderate periportal fibrosis with some bile