

Glutaric Aciduria Type II: Evidence for a Defect Related to the Electron Transfer Flavoprotein or Its Dehydrogenase

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Summary

Incubation of intact fibroblasts from a patient with glutaric aciduria type II with [2-¹⁴C]riboflavin showed normal synthesis of flavin mononucleotide and flavin adenine dinucleotide. This is taken as evidence for normal transport of riboflavin into the cells and normal activity of riboflavin kinase (EC 2.7.1.26) and flavin mononucleotide adenyltransferase (EC 2.7.7.2).

The ability of intact fibroblasts to oxidize 1-¹⁴C-fatty acids and [6-¹⁴C]lysine is impaired in the patient which together with the urinary excretion pattern of organic acids indicates a defective dehydrogenation of fatty acid acyl-CoAs and glutaryl-CoA. However, dehydrogenation of (C₆-C₁₀) fatty acid acyl-CoA derivatives and glutaryl-CoA was normal when the dehydrogenases were measured in fibroblast homogenate with artificial electron acceptors. *In vivo*, these dehydrogenases transfer their electrons to CoQ₁₀ in the main electron transport chain via electron transfer flavoprotein and electron transfer flavoprotein dehydrogenase. Glutaric aciduria type II fibroblasts showed very diminished activity when the glutaryl-CoA dehydrogenase activity was measured without artificial electron acceptor but with intact endogenous electron transport system. As the NADH and succinate oxidation seems normal in glutaric aciduria type II patients, this is strong evidence for a defect in either the electron transfer flavoprotein or the electron transfer flavoprotein dehydrogenase.

Abbreviations

DCPIP, 2,6-dichlorophenolindophenol
 DPBS, Dulbecco's phosphate-buffered saline
 EA, electron acceptor
 ETF, electron transfer flavoprotein
 ETF-DH, electron transfer flavoprotein dehydrogenase
 ETC, electron transport chain
 ETS, electron transport system (*i.e.* ETF, ETF-DH, and the ETC from CoQ₁₀ to O₂)
 GA I, glutaric aciduria type I
 GA II, glutaric aciduria type II
 GADH, general acyl-CoA dehydrogenase
 GDH, glutaryl-CoA dehydrogenase
 MB, methylene blue

The neonatal form of GA II is a serious metabolic disorder with symptoms starting within the first days of life (3, 7, 8, 11, 14). The infants with this disorder become lethargic and hypotonic, they develop severe hypoglycemia and acidosis. Death usually occurs a few days after onset of symptoms. Biochemically,

the disease is characterized by the urinary excretion of a number of organic acids that all seem to be derived from substrates to a group of FAD containing acyl-CoA dehydrogenases (8, 11). Some of the patients have also been noted to have high plasma sarcosine and to excrete elevated amounts of sarcosine in the urine (7, 8). This abnormal metabolic pattern is explained by deficiency of the enzyme systems of the fatty acid acyl-CoA dehydrogenation, the acyl-CoA dehydrogenation involved in the degradation of the three branched chain amino acids, the glutaryl-CoA dehydrogenation and the sarcosine dehydrogenation (8).

A normal synthesis of FAD by kidney homogenate from one patient indicated the presence of normal amounts of this coenzyme for all the involved dehydrogenases (7).

Fibroblasts from patients with GA II have shown diminished ability to oxidize ¹⁴C-labeled substrates for the flavoprotein acyl-CoA dehydrogenases (11, 12).

A defect in ETF common for all the defective dehydrogenases has been suggested by several investigators including ourselves (7, 8). Recently, the ETF has been measured in fibroblasts from the first reported patient with glutaric aciduria type II and found to be normal or increased (12). The present work describes the investigations performed on cultured skin fibroblasts from our patient with GA II to further characterize the defect. Normal uptake of [2-¹⁴C]riboflavin and normal synthesis of FMN and FAD was found. The oxidation of (C₆-C₁₆) 1-¹⁴C-fatty acids and [6-¹⁴C]lysine to ¹⁴CO₂ was significantly decreased in intact fibroblasts from the patient. The glutaryl-CoA and medium chain (C₆-C₁₀) acyl-CoA dehydrogenases in fibroblast homogenate were normal using artificial electron acceptors. However, glutaryl-CoA dehydrogenase activity was very much decreased with intact ETC. These results are consistent with the proposed defect of ETF or ETF-DH.

MATERIALS AND METHODS

The chemicals used were obtained from the following sources: [1,5-¹⁴C]glutaric acid and DL-[6-¹⁴C]lysine from CIS (France, Belgium, and Italy); 1-¹⁴C-labeled even-numbered fatty acids from New England Nuclear; [2-¹⁴C]riboflavin from Amersham, England; riboflavin, FMN, FAD, and CoA from Boehringer, Germany; DCPIP, MB, and trichloroacetic acid from Merck, Germany; methylbenzethonium hydroxide (Hyamin) from Sigma; L-lysine HCl from Calbiochem, Switzerland; fetal calf serum from Gibco, Scotland; medium 199, DPBS, and Hanks balanced salts from Flow Laboratories, Scotland; and Insta-Fluor and Insta-Gel from Packard Instrument Company.

Assays for glutaryl-CoA dehydrogenase. The first method to

determine GDH activity used MB as EA as earlier described (1, 2; Fig. 1). The second method measured GDH activity without exogenous EA and was dependent on the intact endogenous ETS. Different homogenizing procedures were investigated with this method. The procedure that gave the highest activity in normal fibroblasts was chosen as the method for investigating the activity in fibroblasts from GA II patients. The first method determined GDH activity alone whereas the second method also measured the endogenous ETS (see Fig. 2). The second method was thus able to detect defects in GDH as well as in ETS. All assays were performed in duplicate. The values shown in Tables 5, 6, and 7 were the results of three or more separate determinations.

Estimation of the oxidation rate for [6-¹⁴C]lysine. Oxidation of [6-¹⁴C]lysine was performed by a method modified from Dancis *et al.* (4). Fibroblasts were grown to confluence in 25-cm² flasks. The cells were washed three times with 5 ml DPBS with the cells still attached to the bottom. 0.5 ml DPBS containing 0.33 mmol/liter [6-¹⁴C]lysine (specific activity, 2.5. mCi/mol) was added and the flasks were incubated at 37°C for 4 h. Scintillation flasks containing a piece of pleated filter paper moistened with 100 μ l Hyamin were attached to the culture flasks by means of a piece of rubber tubing during incubation. The incubation was stopped by injection of 100 μ l of 30% trichloroacetic acid into the culture flasks through the rubber tubing. The released ¹⁴CO₂ was collected, while shaking the flasks gently at 37°C for a further 60 min. 10 ml Insta-Fluor was added to each scintillation flask and the amount of ¹⁴CO₂ was counted in a Betasint scintillation counter. The cell monolayer was dissolved in a strong base and aliquots were used for protein determination using the Lowry method (10).

Oxidation of 1-¹⁴C-fatty acids (C₆-C₁₆). The determination of the rate of fatty acid oxidation was performed as earlier described (9).

Dehydrogenation of medium chain acyl-CoA (C₆-C₁₀). Measurement of acyl-CoA dehydrogenases in fibroblast homogenate

was performed by selected ion monitoring gas chromatography/mass spectrometry of the 3-OH-acids formed from the reaction products after enzymatic hydration of the unsaturated acyl-CoA derivatives by purified crotonase and hydrolysis of the thioesters. The method has been described in detail elsewhere (9).

FAD and FMN synthesis in fibroblasts: Method I. Fibroblasts were grown to confluence in 75-cm² Falcon Flasks in medium 199 containing 15% fetal calf serum. For measuring riboflavin uptake and conversion, the growth medium was replaced by medium 199 without fetal calf serum but containing 20 μ mol/liter [2-¹⁴C]riboflavin (specific activity, 60 mCi/mmol). After incubation for 24 h at 37°C, the incubation was stopped and cells washed three times with 10 ml DPBS. The cells were then harvested with trypsin-EDTA and further washed twice with DPBS. 3 ml of a solution containing 0.033 mg/ml riboflavin, FMN, and FAD were added. The cells were homogenized in a Potter-Elvehjem homogenizer, subjected to three cycles of freezing and thawing, and finally sonified 3 \times 10 sec on a MSE 150-W Sonifier. An aliquot was used for protein determination. 6 ml methanol was added to the rest of the homogenate and proteins were allowed to precipitate at 4°C for 18 h in the dark. The precipitate was pelleted by centrifugation for 30 min at 3000 \times g. 5 ml of the supernatant was evaporated to dryness in a stream of nitrogen. The residue was redissolved in 2 ml distilled water and applied to a DEAE-Sephadex A-25 column. Riboflavin and its derivatives were then eluted by 1% ammonium sulfate as described by Fazekas and Sandor (6).

Fractions containing the individual flavins were pooled and a fraction was mixed with Insta-gel and counted in a liquid scintillation counter. The intracellular concentrations of labeled riboflavin, FMN, and FAD were expressed as cpm/mg protein.

Method II. Fibroblasts were grown to confluence in 75-cm² Falcon flasks in medium 199 containing 15% FCS. The medium was then replaced by 0.040 μ mol/liter [2-¹⁴C]riboflavin (specific activity, 60 mCi/mmol) dissolved in Hanks' balanced salt solution. The cells were incubated for 4 h at 37°C. The measurement of the intracellular concentration of labeled riboflavin, FMN, and FAD was then performed as for method I.

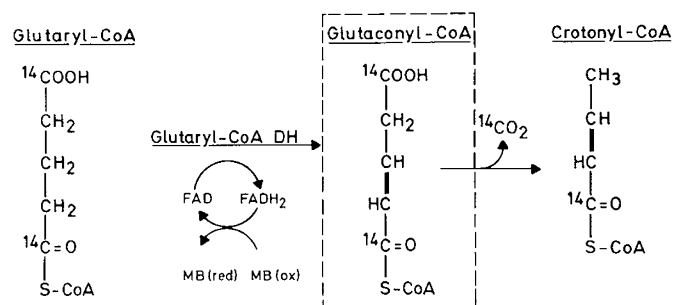


Fig. 1. Glutaryl-CoA dehydrogenation with MB as exogenous electron acceptor.

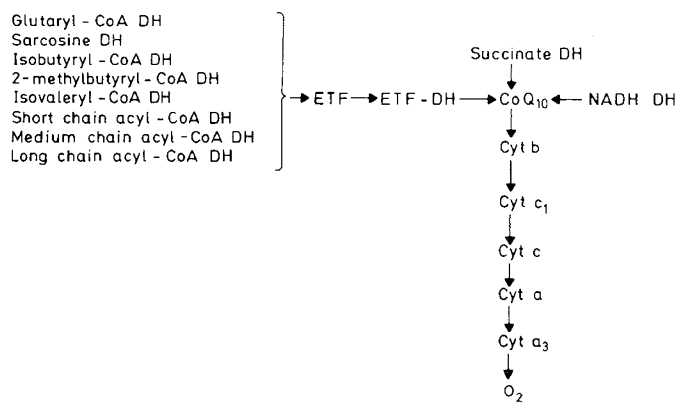


Fig. 2. The electron transport to the respiratory chain for the ETF- and ETF-DH-dependent dehydrogenases.

RESULTS

The ability of the mutant fibroblasts to take up [2-¹⁴C]riboflavin and convert it to labeled FMN and FAD was similar to that of fibroblasts from normal individuals (Table 1). This was the case both at a saturated [2-¹⁴C]riboflavin concentration, where an increase in [2-¹⁴C]riboflavin did not result in a further increase in FAD synthesis and at a low [2-¹⁴C]riboflavin concentration with no saturation of FAD synthesis.

Table 2 shows the oxidation rates for 1-¹⁴C-labeled even-numbered fatty acids (C₆-C₁₆) by intact fibroblasts from normal individuals and the patient. A significant decrease in the ability to oxidize 1-¹⁴C-fatty acids is seen for the mutant fibroblasts.

The oxidation rates of DL-[6-¹⁴C]lysine by different mutant cell lines is compared to cell lines from normal individuals in Table 3. The lowest oxidation rate is seen in two patients with hyperlysinemia. The oxidation rate in the patient with GA II is

Table 1. Incorporation of [2-¹⁴C]riboflavin into FMN and FAD by intact fibroblasts from the patient and from two normal individuals*

	24-h incubation with 20 μ mol/liter [2- ¹⁴ C]riboflavin (cpm/mg protein)		4-h incubation with 0.040 μ mol/liter [2- ¹⁴ C]riboflavin (cpm/mg protein)	
	FMN	FAD	FMN	FAD
Control 1	695	7521	0	1007
Control 2	578	5316	0	1210
Patient	475	4906	0	911

* Each value is the mean of three separate determinations.

Table 2. Oxidation of 1-¹⁴C-fatty acids by intact fibroblasts expressed as pmol/min/mg protein*

	Hexanoic acid	Octanoic acid	Decanoic acid	Lauric acid	Myristic acid	Palmitic acid
Mean ± SD and range in controls	1.26 ± 0.29 (n = 6) (1.03–1.75)	0.60 ± 0.20 (n = 6) (0.38–0.83)	1.81 ± 0.50 (n = 6) (1.20–1.24)	1.62 ± 0.45 (n = 5) (0.97–2.07)	1.23 ± 0.27 (n = 5) (1.02–1.68)	4.78 ± 1.0 (n = 6) (3.61–6.81)
Activity in the patient with glutaric aciduria type II	0.34	0.07	0.26	0.16	0.23	0.90
P†	<0.025	<0.025	<0.025	<0.025	<0.025	<0.01

* For the patient, each value is the mean of a duplicate determination.

† Student's *t* test (one-tailed) was used to compare activities in cells from the patient with activity in control cells.

Table 3. Oxidation of [6-¹⁴C]lysine by fibroblasts from patients with glutaric aciduria types I and II and from patients with hyperlysinemia with and without saccharopinuria and from normal individuals*

Patient	¹⁴ CO ₂ evolved (pmol/min/mg protein)	% of control
Control†	0.45 (0.39–0.52)	100
Glutaric aciduria type I	0.21	47
Glutaric aciduria type II	0.20	44
Patient with hyperlysinemia	0.08	18
Patient with hyperlysinemia and saccharopinuria	0.04	9

* For each individual, the value is the mean of two determinations.

† The activity is the mean of the activity found in three normal individuals. The range is in parentheses.

about half the normal rate and similar to the rate in patients with GA I.

Medium chain acyl-CoA dehydrogenase activity estimated by direct dehydrogenation of (C₆–C₁₀) acyl-CoA by fibroblast homogenate is shown in Table 4. Normal activity was found in the homogenates of GA II fibroblasts. Glutamate dehydrogenase was determined as a reference mitochondrial enzyme. The normal dehydrogenation of (C₆–C₁₀) acyl-CoAs by fibroblast lysate from GA II patients is in contrast to the very decreased oxidation by intact cells of (C₆–C₁₆) fatty acids as seen in Table 2 and indicates that the dehydrogenases are functioning normally.

In order to investigate the influence of the homogenizing procedure, GDH activities have been measured in normal fibroblasts both with MB and without any exogenous EA, but with intact endogenous ETS after five different homogenizing procedures as shown in Table 5. The highest activity with MB as EA was obtained with procedure I. This was comparable to the previously reported procedure (2). The measured activity with MB as EA decreased when part of the procedure was omitted, and less than 10% of normal activity when only one stroke of a Potter-Elvehjem homogenizer was used. The GDH activity with no exogenous EA but with intact endogenous ETS on the contrary increased with less vigorous homogenizing procedure. Maximum activity was obtained with one freeze-thaw cycle after Potter-Elvehjem homogenizing. Without the freeze-thaw cycle, the activity decreased as did the activity with MB as EA. Centrifugation of the fibroblast homogenate for 1 hr at 50,000 × *g* completely abolished the glutaryl-CoA dehydrogenase activity without exogenous EA as did the addition of 0.1% Triton X-100, while the MB-dependent activity was unchanged. In agreement with the results from Table 5, homogenizing procedure IV was used for the determination of GDH activity with endogenous EA (*i.e.* intact ETC).

In Table 6 are shown the GDH activities in normal fibroblasts measured with homogenizing procedure 4 from Table 5 after the addition of compounds known to inhibit the ETC. GDH activity

is inhibited by both KCN and antimycin A. The activities with only endogenous EA are inhibited most, indicating that the GDH activities with no exogenous EA are dependent on an intact ETC for activity.

The GDH activities in different fibroblast cell lines are shown in Table 7 both with endogenous EA and with MB as EA. The activities in normal individuals with MB as EA are lower than previously reported (2). This is caused by the suboptimal homogenizing procedure for this method.

GA I heterozygotes showed the same ratio as normal homozygotes between the activity measured with endogenous EA and the activity with MB as EA (see last column in Table 7). GA I homozygotes have no activity with either endogenous ETS or MB as EA whereas GA II homozygotes have normal activity measured with MB as EA but very low activity with endogenous EA (*i.e.* with intact ETC). In contrast to GA I heterozygotes with half-normal activity with both assay methods, GA II heterozygotes cannot be distinguished from normal homozygotes by these two methods.

DISCUSSION

All cases of neonatal GA II described have been characterized by the urinary excretion of a great number of organic acids that all seem to be derived from substrates to a group of FAD-containing acyl-CoA dehydrogenases (8). Besides FAD, these dehydrogenases have in common a transfer of electrons through ETF and ETF-DH to CoQ₁₀ in the ETC (see Fig. 2). The normal activities of the fatty acyl-CoA dehydrogenases and of GDH measured on fibroblast homogenate with artificial electron acceptors strongly indicate that the dehydrogenases themselves are functioning normally in GA II.

A defect in a common factor for all of the involved dehydrogenases has been considered. One such factor is FAD, the common prosthetic group for all the apparently involved enzymes. A decreased synthesis of FAD as already suggested by Przyrembel *et al.* (11) could be the cause of the defective function of the dehydrogenases *in vivo*. Goodman *et al.* (7) investigated the ability of an autopsy kidney specimen from one of his patients with GA II to synthesize FMN and FAD from [2-¹⁴C]riboflavin and found no difference between autopsy kidney from the patient and three controls. As has been reported that whole liver homogenate only has slight net synthesis of FMN and FAD due to degradation by factors contained in the mitochondria (5), we decided to reinvestigate the FMN and FAD production from [2-¹⁴C]riboflavin in our patient using whole living fibroblasts as the tissue for investigation.

Two different methods were used: one using 24-h incubation in normal medium and a high [2-¹⁴C]riboflavin concentration and one using 4-h incubation in a salt solution containing glucose but without unlabeled riboflavin and a rather low [2-¹⁴C]riboflavin concentration not saturating the FMN- and FAD-synthesizing system. The latter system should be able to detect a defective uptake of riboflavin by the mutant cells. Both methods showed normal FAD synthesis by the mutant cells, thus confirming the results by Goodman *et al.* (7) that FAD synthesis is normal in patients with GA II.

ETF and ETF-DH are essential factors for the FAD-containing

Table 4. Dehydrogenase activity in fibroblast homogenate expressed as nmol/h/mg protein*

	Substrate			
	Hexanoyl-CoA	Octanoyl-CoA	Decanoyl-CoA	Glutamate†
Mean \pm SD and range in controls	18.2 \pm 6.4 (n = 5) (11.0–24.4)	30.0 \pm 12.1 (n = 5) (15.0–42.1)	22.3 \pm 5.8 (n = 5) (12.9–28.3)	26.2 \pm 4.8 (n = 4) (19.3–29.9)
Glutaric aciduria type II patient	22.6	27.7	26.2	23.0

* For the patient, each value is the mean of a duplicate determination.

† nmol/min/mg protein.

Table 5. The influence of the homogenizing procedure on the glutaryl-CoA dehydrogenase activity both with MB and with endogenous electron acceptors

Homogenizing procedure	Glutaryl-CoA dehydrogenase activity (μ mol/h/g protein)		100 \times B A
	With MB as EA (A)	With endogenous EA (B)	
I. Potter-Elvehjem homogenizing 1 freeze-thaw cycle, 0.1% Triton X-100	4.86	0.008	0.16
II. Potter-Elvehjem homogenizing 3 freeze-thaw cycles, sonification for 3 \times 10 sec	4.65	0.14	2.17
III. Potter-Elvehjem homogenizing, 3 freeze-thaw cycles	3.98	0.58	14.6
IV. Potter-Elvehjem homogenizing, 1 freeze-thaw cycle	3.07	0.85	27.7
V. Potter-Elvehjem homogenizing	0.31	0.09	29.0

Table 6. Inhibition of glutaryl-CoA dehydrogenase activity by KCN and antimycin A using both MB and endogenous electron acceptors

Assay mixture	Glutaryl-CoA dehydrogenase activity (μ mol/h/g protein)		100 \times B A
	With MB as EA (A)	With endogenous EA (B)	
Normal	2.01	0.32	15.9
+10 mmol/liter KCN	0.76	0.02	2.6
+2 mg/liter antimycin A	1.49	0.10	6.7

Table 7. The activity of glutaryl-CoA dehydrogenase with both MB and endogenous electron acceptors in a normal homozygote, GA I and GA II heterozygotes, and homozygotes

Fibroblast cell line	Glutaryl-CoA dehydrogenase activity (μ mol/h/g protein)		100 \times B A
	With MB as EA (A)	With endogenous EA (B)	
Normal homozygote	2.86	0.44	15.4
GA I heterozygote	1.34	0.24	17.9
GA I homozygote	0.00	0.00	
GA II heterozygote, mother	1.96	0.37	18.9
GA II heterozygote, father	2.99	0.53	17.7
GA II homozygote	2.68	0.014	0.5

acyl-CoA dehydrogenases to be able to deliver electrons to CoQ₁₀ and thereby to the main ETC. A defect in either one of these factors would abolish the *in vivo* activity for all the acyl-CoA dehydrogenases. A defective ETF common to all the deficient dehydrogenases has been suggested by several investigators including ourselves (7, 8). Recently, the ETF has been measured

by Rhead *et al.* (12) in fibroblasts from the first reported patient with GA II and found to be normal or increased. He used a system with excess pig liver GADH, octanoyl-CoA as substrate and DCPIP as the terminal EA. This system requires an additional EA for transfer of electrons from GADH to DCPIP such as phenazine methosulfate or naturally occurring ETF. Rhead *et al.* (personal communication) used fibroblast mitochondrial sonicate as the ETF source and assumed the reduction in DCPIP to be a measure of the ETF activity. However, the fibroblast mitochondrial sonicate contains thiolases which hydrolyze octanoyl-CoA and liberate thiol groups that are able to reduce DCPIP (12).

As ETF and ETF-DH activity seems difficult to measure in fibroblasts, we have tried to obtain an indirect measure of their combined activity by measuring GDH with intact ETS and without exogenous EA. GDH activity is then dependent on both a normal GDH, ETF, ETF-DH, and an intact ETC. GA II homozygotes have very low activity using this method whereas they have normal activity with MB as EA. These results strongly indicate a normal GDH apoenzyme but that one of the components in the electron transport chain from GDH to molecular oxygen is defective. As the oxidation of NADH and succinate seems to be normal in GA II, the ETC from CoQ₁₀ to O₂ is very likely also intact (13). The possible defect in GA II is then limited to ETF or ETF-DH.

Neonatal GA II has been suggested to be inherited as an X-linked disease (3). The support for this has been that most of the patients found so far have been male infants. However, the case described by us who had the severe neonatal form of the disease was a female. This leads to different possibilities. The severe neonatal form can be a heterogenous group with more than one biochemical defect, one being X-linked and one autosomal recessively inherited. A way to distinguish between these possibilities would be to show half-normal activity in the GA II parents. As the exact defect in GA II is not known at present, this must be done indirectly. The oxidation of fatty acids in intact GA II fibroblasts is very much decreased (see Table 2). If intermediate oxidation rates of fatty acids could be shown in parents of GA II patients, this would strongly support a recessive inheritance. Our method that measures GDH with intact ETC as EA to diagnose

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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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 ($26.2 \pm 1.3\%$) 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