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Comparison of kinetic study of the photochemical changes of (*ZZ*)-bilirubin IXα bound to human serum albumin with that bound to rat serum albumin

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It has been stated by McDonagh, Palma & Lightner [(1982) *J. Am. Chem. Soc.* **104**, 6867–6871] that complexing of bilirubin with serum albumin has a marked species-dependent influence on bilirubin photoisomerization *in vitro* and *in vivo*. Therefore the kinetics for the quantitatively important reaction:

$$(EZ)\text{-Cyclobilirubin} \xleftarrow{k_5} (EZ)\text{-bilirubin} \underset{k_3}{\overset{k_4}{\rightleftharpoons}} (ZZ)\text{-bilirubin} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (ZE)\text{-bilirubin}$$

of the photochemical interconversion between bilirubin and its photoisomers bound to human or rat serum albumin in aqueous solution, assayed by h.p.l.c., was used to elucidate the observed species-dependent difference. The relative rate constants for bilirubin bound to human serum albumin, except for k_4, the rate of interconversion from (*ZZ*)-bilirubin into (*EZ*)-bilirubin, proved to be considerably larger than those for bilirubin bound to rat serum albumin. In accordance with these rate constants, the formation of photoisomers of bilirubin bound to human serum albumin, except for (*EZ*)-bilirubin, is very rapid and much greater than that for bilirubin bound to rat serum albumin.

The photochemistry of (*ZZ*)-bilirubin and the chemical mechanism of bilirubin removal from the body during phototherapy are very important because of the widespread clinical use of phototherapy for treating neonatal hyperbilirubinaemia (Brown & McDonagh, 1980). Although mechanistic studies of phototherapy have been carried out in humans (Onishi *et al.*, 1980*b*; Lamola *et al.*, 1981; Ennever *et al.*, 1983), most studies have been performed with the Gunn rat animal model of the human neonatal infant (Ostrow, 1971; McDonagh *et al.*, 1980; McDonagh & Palma, 1980; Stoll *et al.*, 1981; Onishi *et al.*, 1981, 1984*b*). However, McDonagh *et al.* (1982*b*) have stated that complexing of (*ZZ*)-bilirubin with serum albumin has a marked species-dependent influence on bilirubin photoisomerization, and in particular on the regioselectivity of configurational isomerization. For example, configurational isomerization of (*ZZ*)-bilirubin bound to horse, rat, guinea-pig or bovine serum albumin generates (*EZ*)- and (*ZE*)-bilirubins, with a slight preference for the latter isomer. In contrast, (*ZZ*)-bilirubin bound to human serum albumin yields, under the same conditions, only one of the two diastereomeric *E*/*Z* isomers, (*ZE*)-bilirubin, although it is considered that photochemically the two chromophoric systems behave as independent units (de Groot *et al.*, 1982). This would lead one to predict that similar marked stereoselectivity and regioselectivity may be observed in human neonatal infants during phototherapy, but not in the Gunn rat. In fact, as described previously, the main biliary and urinary bilirubin photoisomers excreted by human neonatal infants during phototherapy are structural isomers, i.e. (*EZ*)-cyclobilirubin (Onishi *et al.*, 1980*a*,*b*, 1984*a*), and the main biliary and urinary bilirubin photoisomers excreted by Gunn rats during phototherapy are geometric isomers, i.e. (*EZ*)- and (*ZE*)-bilirubins (Onishi *et al.*, 1984*b*). Thus the photochemistry observed *in vivo* appears to resemble that observed *in vitro* very closely. Therefore in the present investigation the species-dependent differences in the kinetics of the photochemical interconversion between bilirubin and its photoisomers bound to human or rat serum albumin in aqueous solution were determined by h.p.l.c.

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Materials and methods

Photochemical experiments

Several portions of the solution in Pyrex tubes, 0.5ml for photochemical reaction and 0.2ml for kinetic study, were exposed to light. At different light exposures, the Pyrex tubes were successively transferred to the dark. Each portion was analysed by h.p.l.c. The light intensity at the surface of the solution, determined with a Minolta Air-Shields Fluoro-lite-meter 451 (Minolta Air-Shields), was $27.3\,W\cdot cm^{-2}\cdot nm^{-1}$. Except for photochemical experiments, all manipulations were carried out in the dark or photographic safe-light, and at room temperature.

Preparation for photochemical experiments

Photochemical reaction. Solutions of human or rat serum albumin and bilirubin (100μg/ml) were prepared by adding 0.1ml of a solution, obtained by dissolving 5mg of crystalline bilirubin in 0.5ml of 0.05M-NaOH, to 10ml of human serum albumin solution (20mg/ml) or rat serum albumin solution (20mg/ml) in 0.1M-sodium phosphate buffer, pH7.4.

Kinetic study. (1) Solutions of (*ZZ*)-bilirubin rat serum albumin. Solutions of rat serum albumin and bilirubin (43.5μg/ml) were prepared as follows. Crystalline bilirubin (10mg) was dissolved in 10.0ml of 0.05M-NaOH, and 50μl of the solution was added to 1.0ml of rat serum albumin solution (20mg/ml) in 0.1M-sodium phosphate buffer, pH7.4. The mixture was further diluted with 100μl of the same buffer.

(2) Solutions of human or rat serum albumin enriched with (*EZ*)- and (*ZE*)-bilirubins. Preparations highly enriched in (*EZ*)- and (*ZE*)-bilirubins (approx. 50μg/ml) were obtained by solvent extraction of crude photolysis products. A trace of EDTA was added to a saturated bilirubin solution in distilled chloroform. The solution was deoxygenated with pure (99.99%) N_2 for 3min. Under anaerobic conditions, four 2.0ml portions of the solution, in stoppered 10ml Pyrex glass tubes located 3cm above the canopy of four fluorescent tubes, were exposed to light for 10min. The residues obtained after evaporation of the solvents during centrifugation *in vacuo* at room temperature were mixed with methanol. The purpose of the next procedure is to avoid contamination with (*ZZ*)-bilirubin as much as possible. The supernatant obtained from centrifugation at 7000*g* for 5min was filtered rapidly through a membrane filter. The residues obtained from the supernatant by a similar evaporation procedure were dissolved in 400μl of 0.1M-sodium phosphate buffer, pH7.4, and 150μl of the solution was added to 1.0ml of human or rat serum albumin solution (20mg/ml) and then kept in the dark at 4°C for 24h. The supernatant obtained from centrifugation at 7000*g* for 5min was used for the photochemical experiment.

Sample preparation for h.p.l.c.

Dimethyl sulphoxide (1vol.) and acetonitrile (1vol.) were added to 1vol. of the sample, and the whole was vortex-mixed for 30s and then centrifuged at 7000*g* for 5min. A 25μl portion of the supernatant was injected into the chromatograph. The preparation was carried out within 6min immediately before injection.

Equipment, preparation of the calibration curve, peak assignments and pigments and reagents were as described previously (Onishi *et al.*, 1979, 1980*a*; Isobe & Onishi, 1981; Itoh *et al.*, 1983). Peak assignments of (*EZ*)- and (*ZE*)-bilirubins were carried out as previously described by McDonagh *et al.* (1982*a*). Chromatographic operation was also as described previously (Itoh & Onishi, 1985).

Results and discussion

Photochemical changes of bilirubin bound to serum albumin

(1) *Human serum albumin* (*Fig. 1*). When photoirradiation was applied, (*ZZ*)-bilirubin concentration decreased, initially rapidly and subsequently slowly with a half-life of 16.2min, whereas (*ZE*)-bilirubin appeared abruptly and then decreased. The slopes of the disappearance curves of these two peaks were parallel. The ratio of (*ZE*)-bilirubin to (*ZZ*)-bilirubin was 0.50:1. At the same time, (*EZ*)- and (*EE*)-cyclobilirubins and (*EE*)-bilirubin appeared, initially rapidly, and then increased slowly. The changes in the percentages of the individual photoisomers and parent pigment (*ZZ*)-bilirubin after the initiation of photoirradiation were as shown in Table 1.

(2) *Rat serum albumin* (*Fig. 2*). When photoirradiation was applied, (*ZZ*)-bilirubin concentration decreased with a half-life of 44.8min, which is very long, i.e. a very slow photochemical change compared with that in the presence of human serum albumin. Not only (*ZE*)-bilirubin but also (*EZ*)-bilirubin appeared abruptly and then decreased in parallel. The ratio of (*ZE*)-bilirubin to (*ZZ*)-bilirubin was 0.08:1, which is very low compared with 0.5:1 for human serum albumin. The changes in the percentages of the individual photoisomers and parent pigment after initiation of photoirradiation were as shown in Table 1.

Thus the identity of the serum albumin had a marked effect on the rate of photoisomer formation and on the composition of the photoproducts. This agrees with a previous report (McDonagh *et al.*, 1982*a*,*b*). Because photodecomposition via

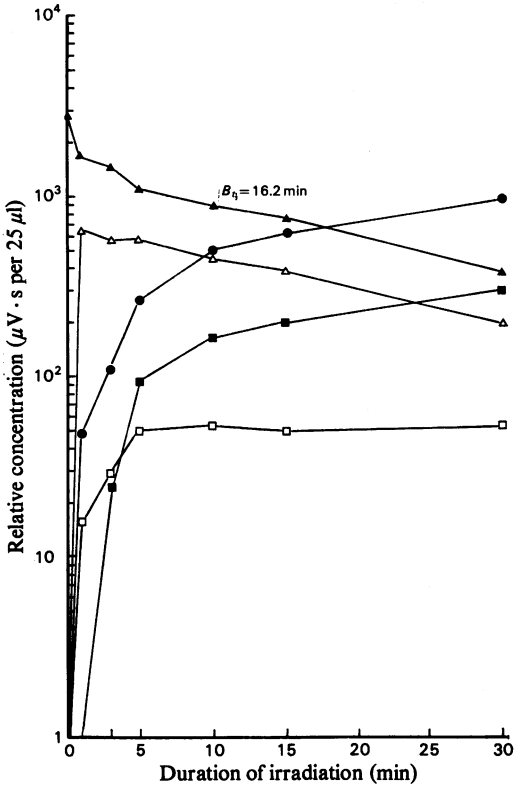

Fig. 1. *Changes in the relative concentrations of bilirubin and its photoisomers plotted against time on a semi-logarithmic scale as a result of photochemical reaction after irradiation of (ZZ)-bilirubin bound to human serum albumin*

The half-life of (*ZZ*)-bilirubin (B) bound to human serum albumin for the slow reaction occurring after 5 min of irradiation is 16.2 min. Experimental details are given in the text. Symbols: ▲, (*ZZ*)-bilirubin; △, (*ZE*)-bilirubin; ●, (*EZ*)-cyclobilirubin; ■, (*EE*)-cyclobilirubin; □, (*EE*)-bilirubin. For Figs. 1 and 2, 4.2×10^5 μV·s is equivalent to 1.0 μg of bilirubin.

(*EZ*)-cyclobilirubin may occur gradually (von Dobeneck, 1979; Onishi *et al.*, 1982), strictly speaking no quantitative correspondence between the disappearance of (*ZZ*)-bilirubin and the appearance of the photoisomers in serum albumin solution was observed.

Kinetic study of photochemical interconversion of bilirubin and its photoisomers bound to rat serum albumin

(1) *(ZZ)-Bilirubin bound to rat serum albumin.* (*a*) Photoirradiation for a short time (Fig. 3). When photoirradiation was applied for a short time (30 s), the peaks of (*EZ*)- and (*ZE*)-bilirubins appeared rapidly and the peaks of (*EZ*)- and (*EE*)-cyclobilirubins and of (*EE*)-bilirubin remained negligible,

Table 1. *Relative concentrations of bilirubin and its photoisomers produced by photoirradiation of bilirubin bound to human or rat serum albumin*

For details see Itoh & Onishi (1985).

Duration of irradiation (min)	Relative concentration (%)						
	(*EE*)-Cyclobilirubin	(*EE*)-Bilirubin	(*EZ*)-Cyclobilirubin (C)	(*ZE*)-Bilirubin (D)	(*EZ*)-Bilirubin (A)	(*ZZ*)-Bilirubin (B)
Human serum albumin (Itoh & Onishi, 1985)						
0						100
1	0	0.7	2.0	27.2	0	70.1
3	1.1	1.1	4.9	25.9	0	67.0
5	1.4	0.7	8.8	25.0	0	64.1
10	7.7	2.5	22.9	22.1	0	43.9
15	9.9	2.4	30.9	18.8	0	38.0
30	15.8	2.8	51.1	10.4	0	19.9
Rat serum albumin						
0						100
1	0	0.6	0	7.5	3.1	88.8
3	0.7	0.8	0.8	7.3	3.5	86.9
5	1.2	0.9	1.3	6.8	3.8	86.0
10	2.1	1.0	3.8	6.6	3.1	83.4
15	2.1	1.1	4.4	6.1	3.2	83.1
30	5.6	1.2	12.3	5.4	2.7	72.8

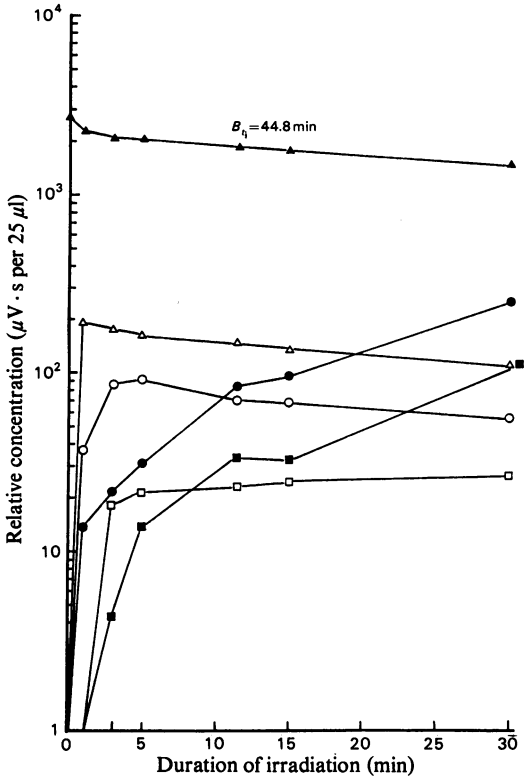

Fig. 2. *Changes in the relative concentrations of bilirubin and its photoisomers plotted against time on a semi-logarithmic scale as a result of photochemical reaction of (ZZ)-bilirubin bound to rat serum albumin*
The half-life of (*ZZ*)-bilirubin (B) bound to rat serum albumin for the slow reaction occurring after 5 min of irradiation is 44.8 min. For details see the text. Symbols: ○, (*EZ*)-bilirubin; others as defined in the legend to Fig. 1.

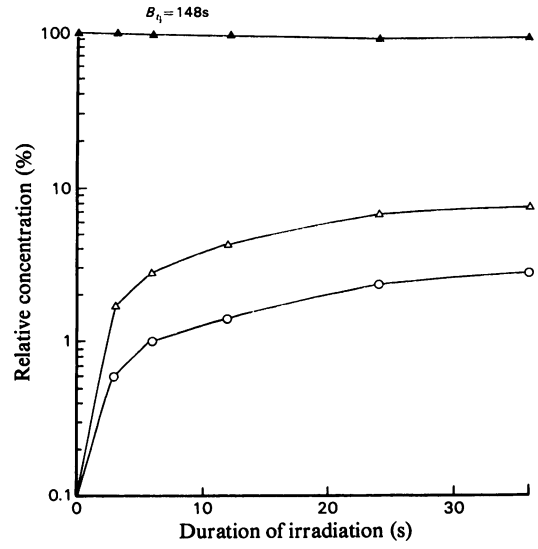

Fig. 3. *Changes in percentages of (ZZ)-bilirubin and (EZ)-bilirubin and (ZE)-bilirubin as a result of photochemical isomerization after irradiation of (ZZ)-bilirubin bound to rat serum albumin for a short time*
The half-life of (*ZZ*)-bilirubin (B) bound to rat serum albumin is 148 s. For details see the text. Explanation of the symbols is given in the legends to Figs. 1 and 2.

whereas the peak of (*ZZ*)-bilirubin decreased exponentially up to 12 s with a half-life of approx. 148 s (Fig. 3). Therefore the kinetics:

$$(EZ)\text{-Bilirubin} \xleftarrow{k_4} (ZZ)\text{-bilirubin} \xrightarrow{k_1} (ZE)\text{-bilirubin}$$
$$\text{(A)} \qquad\qquad \text{(B)} \qquad\qquad \text{(D)}$$

will give a close approximation of the required values.

A, *B*, *C* and *D* represent percentages of photoisomers, i.e. A, C and D and their parent pigment B as defined in Tables 1–3. Then:

$$A + B + C + D = 100(\%),\ t = 0,\ B_0 = 100(\%)$$

$$\frac{dB}{dt} = -(k_1 + k_4)B$$

$$B = B_0 e^{-(k_1+k_4)t}$$

$$\frac{dA}{dt} = k_4 B = k_4 B_0 e^{-(k_1+k_4)t}$$

$$A = -\frac{k_4}{k_1+k_4} \cdot B_0 e^{-(k_1+k_4)t} + \frac{k_4}{k_1+k_4} \cdot B_0$$

$$\frac{dD}{dt} = k_1 B = k_1 B_0 e^{-(k_1+k_4)t}$$

$$D = -\frac{k_1}{k_1+k_4} \cdot B_0 e^{-(k_1+k_4)t} + \frac{k_4}{k_1+k_4} \cdot B_0$$

From the graph in Fig. 3:

$$B_{t_{\frac{1}{2}}} = 148(\text{s})$$

$$k_1 + k_4 = \frac{\ln 2}{148} = 4.67 \times 10^{-3}\ (\text{s}^{-1})$$

As $t \to \infty$ (the reaction is considered to have reached photoequilibrium 120 s after the start of irradiation as shown in Table 2):

$$A \to \frac{k_4 B_0}{k_1+k_4} = 4.6$$

$$\therefore k_4 = 4.6 \times \frac{k_1+k_4}{B_0} = 4.6 \times 10^{-2} \times 4.67 \times 10^{-3}$$

$$= 2.15 \times 10^{-4}\ (\text{s}^{-1})$$

Table 2. *Prolonged photochemical isomerization of (ZZ)-bilirubin in the presence of rat serum albumin*
For details see Itoh & Onishi (1985).

Duration of irradiation (s)	Relative concentration (%)			
	(*EZ*)-Cyclobilirubin (C)	(*EZ*)-Bilirubin (A)	(*ZZ*)-Bilirubin (B)	(*ZE*)-Bilirubin (D)
60	0.3	3.7	87.3	8.7
120	0.4	4.6	86.1	8.9
240	1.0	4.7	85.6	8.7
480	2.2	4.8	84.9	8.1
960	4.5	4.8	82.6	8.1

$$D \rightarrow \frac{k_1 B_0}{k_1 + k_4} = 8.9$$

$$\therefore k_1 = 8.9 \times \frac{k_1 + k_4}{B_0} = 8.9 \times 10^{-2} \times 4.67 \times 10^{-3}$$

$$= 4.15 \times 10^{-4}\ (s^{-1})$$

(*b*) Photoisomerization for a prolonged time. Photoirradiation of (*ZZ*)-bilirubin in rat serum albumin solution for 960s produced the changes shown in Table 2. Since the percentages of A, B and D were almost unchanged 120s after the start of irradiation, there is a photoequilibrium of A⇌B⇌D.

After 120s the concentration of A is approximately constant ($A = 4.7\%$). Hence the rate constant k_5 can be determined as follows:

$$\underset{\text{(C)}}{\text{(}EZ\text{)-Cyclobilirubin}} \xleftarrow{k_5} \underset{\text{(A)}}{\text{(}EZ\text{)-bilirubin}}$$

$$\frac{dC}{dt} = k_5 A$$

$$C = k_5 A t$$

From the graph in Fig. 4:

$$k_5 A = 0.276$$

$$k_5 = 0.276 \div 4.7 \div 60 = 9.78 \times 10^{-4}\ (s^{-1})$$

(2) (*EZ*)- *and* (*ZE*)-*Bilirubin-enriched rat serum albumin* (*Fig. 5*). When photoirradiation was applied to the (*EZ*)- and (*ZE*)-bilirubins/rat serum albumin solution for 0, 3, 6, 12 and 24s, formation of (*EZ*)-cyclobilirubin was negligible. For the initial 6s of irradiation, the half-lives of the (*EZ*)- and (*ZE*)-bilirubins were 7.4 and 5.8s respectively (Fig. 5). Therefore, for the initial 6s, the kinetics:

$$\underset{\text{(A)}}{\text{(}EZ\text{)-Bilirubin}} \xrightarrow{k_3} \underset{\text{(B)}}{\text{(}ZZ\text{)-bilirubin}} \xleftarrow{k_2} \underset{\text{(D)}}{\text{(}EZ\text{)-bilirubin}}$$

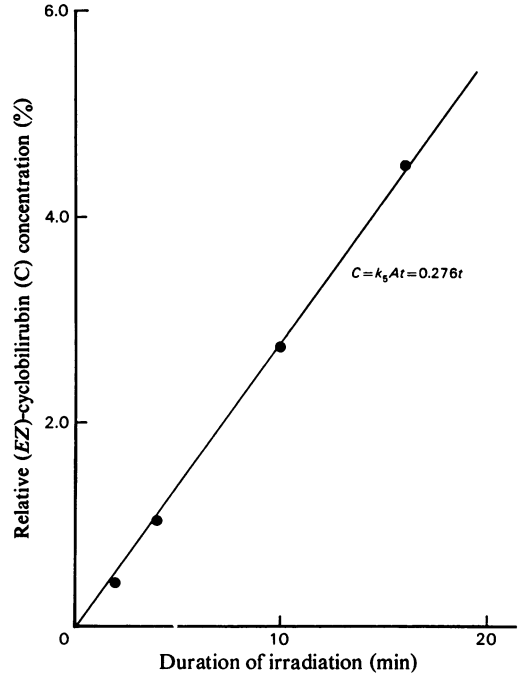

Fig. 4. *Relationship between duration (t) of irradiation and (EZ)-cyclobilirubin (C) concentration formed from (ZZ)-bilirubin bound to rat serum albumin*
The relative (*EZ*)-cyclobilirubin concentration (%) represents changes in percentage of (*EZ*)-cyclobilirubin as a result of photochemical isomerization after irradiation of (*ZZ*)-bilirubin bound to rat serum albumin for a prolonged time. $C = k_5 A t = 0.276t$. For details see the text.

will give a close approximation to the required values:

$$\frac{dA}{dt} = -k_3 A$$

$$A = A_0 e^{-k_3 t}$$

From the graph in Fig. 5:

Table 3. *Summary of the main relative rate constants for the individual photochemical reaction of bilirubin bound to human serum albumin or rat serum albumin*

$$\underset{(C)}{(EZ)\text{-Cyclobilirubin}} \xleftarrow{k_5} \underset{(A)}{(EZ)\text{-bilirubin}} \underset{k_3}{\overset{k_4}{\rightleftharpoons}} \underset{(B)}{(ZZ)\text{-bilirubin}} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{(B)}{(ZE)\text{-bilirubin}}$$

k is the relative rate constant for photochemical reaction of (*ZZ*)-bilirubin to (*EZ*)-cyclobilirubin while bound to human serum albumin, i.e. (Itoh & Onishi, 1985):

$$(EZ)\text{-Cyclobilirubin} \xleftarrow{k} [(EZ)\text{-bilirubin}] \xleftarrow{k} (ZZ)\text{-bilirubin}$$

Rate constant	Human serum albumin	Rat serum albumin
k_1	$3.34 \times 10^{-2}\ s^{-1}$	$4.15 \times 10^{-4}\ s^{-1}$
k_2	$1.06 \times 10^{-1}\ s^{-1}$	$1.20 \times 10^{-2}\ s^{-1}$
k_3	$3.02 \times 10^{-2}\ s^{-1}$	$9.34 \times 10^{-2}\ s^{-1}$
k_4	$7.48 \times 10^{-4}\ s^{-1}$	$2.15 \times 10^{-4}\ s^{-1}$
k_5	$2.76 \times 10^{-2}\ s^{-1}$	$9.78 \times 10^{-4}\ s^{-1}$
	$k \fallingdotseq k_4$ ($\because k \ll k_5$)	

$$A_{t_i} = 7.4\,(s)$$

$$k_3 = \frac{\ln 2}{7.4} = 9.34 \times 10^{-2}\,(s^{-1})$$

$$\frac{dD}{dt} = -k_2 D$$

$$D = D_0 e^{-k_2 t}$$

From the graph in Fig. 5:

$$D_{t_i} = 5.8\,(s)$$

$$k_2 = \frac{\ln 2}{5.8} = 1.20 \times 10^{-2}\,(s^{-1})$$

A summary of the relative rate constants for the individual photochemical reactions of (*ZZ*)-bilirubin bound to human or rat serum albumin is shown in Table 3. Except for k_4 and k_3, the rate constants are considerably larger for human serum albumin. Since the rate constant of the endovinyl cyclization, k_5, step and that of the reversion, k_3, from (*EZ*)-bilirubin to (*ZZ*)-bilirubin are much larger than that of (*EZ*)-bilirubin formation from (*ZZ*)-bilirubin, only (*EZ*)-cyclobilirubin is apparently detected. Thus the marked species-dependent influence of serum albumin on bilirubin photoisomerization (McDonagh *et al.*, 1982*a*,*b*) is explained by the relative rate constants in the individual photochemical steps. Similar differences in relative rate constants may explain the bilirubin photochemistry observed *in vivo* in human neonatal infants and Gunn rats undergoing phototherapy. The reason for the different rate constants for human and rat serum albumins is probably related to differences in bilirubin conformation at the binding site of serum albumin, as suggested by c.d. spectra (Blauer & King, 1970; Kamisaka *et al.*, 1975) and by amino acid sequence studies (Kuenzle *et al.*, 1976; Sjödin *et al.*, 1977; Geisow & Beaven, 1977; Jacobsen, 1978; Sargent *et al.*, 1981). Therefore the kinetics of photochemical reactions may also provide insight into the molecular conformation of the bilirubin binding site in serum albumin.

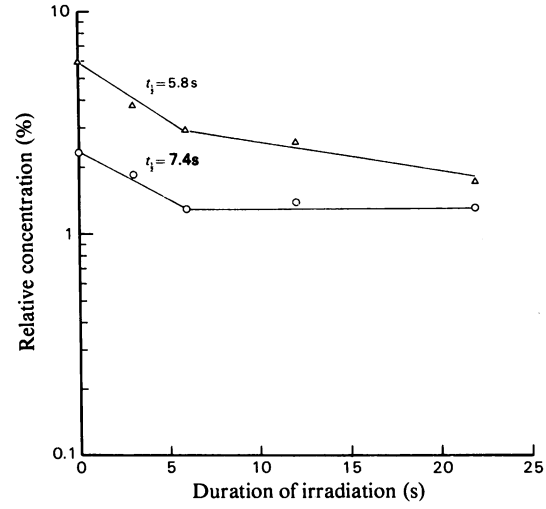

Fig. 5. *Changes in percentages of* (*EZ*)*-bilirubin and* (*ZE*)*-bilirubin as a result of photochemical isomerization after irradiation of both* (*EZ*)*-bilirubin and* (*ZE*)*-bilirubin bound to rat serum albumin*

For the initial 6s of irradiation, half-lives of (*EZ*)-bilirubin (A) and (*ZE*)-bilirubin (D) bound to rat serum albumin are 7.4s and 5.8s respectively. For details see the text. Explanation of the symbols is given in the legends to Figs. 1 and 2.

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References

Blauer, G. & King, T. E. (1970) *J. Biol. Chem.* **245**, 372–381

Brown, A. K. & McDonagh, A. F. (1980) *Adv. Pediatr.* **27**, 341–389

de Groot, J. A., van der Steen, R. & Lugtenburg, J. (1982) *Recl. Trav. Chim. Pays-Bas* **101**, 263–266

Ennever, J. F., McDonagh, A. F. & Speck, W. T. (1983) *J. Pediatr.* **103**, 295–299

Geisow, M. J. & Beaven, G. H. (1977) *Biochem. J.* **161**, 619–625

Isobe, K. & Onishi, S. (1981) *Biochem. J.* **193**, 1029–1032

Itoh, S. & Onishi, S. (1985) *Biochem. J.* **226**, 251–258

Itoh, S., Fujitani, K., Hosoe, A., Yamakawa, T. & Onishi, S. (1983) *Dynamics of Bilirubin Photoisomers in Bilirubin Metabolism during Phototherapy*, Ministry of Health and Welfare of Japan for Research on Prevention of Physical and Mental Disabilities, Tokyo

Jacobsen, C. (1978) *Biochem. J.* **171**, 453–459

Kamisaka, K., Listowsky, I., Gatmaitan, Z. & Arias, I. M. (1975) *Biochemistry* **14**, 2175–2180

Kuenzle, C. C., Gitzelmann-Cumarasamy, N. & Wilson, K. J. (1976) *J. Biol. Chem.* **251**, 801–807

Lamola, A. A., Blumberg, W. E., McClead, R. & Fanaroff, A. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1882–1886

McDonagh, A. F. & Palma, L. A. (1980) *J. Clin. Invest.* **66**, 1182–1185

McDonagh, A. F., Palma, L. A. & Lightner, D. A. (1980) *Science* **208**, 145–151

McDonagh, A. F., Palma, L. A., Trull, F. R. & Lightner, D. A. (1982*a*) *J. Am. Chem. Soc.* **104**, 6865–6867

McDonagh, A. F., Palma, L. A. & Lightner, D. A. (1982*b*) *J. Am. Chem. Soc.* **104**, 6867–6871

Onishi, S., Itoh, S., Kawade, N., Isobe, K. & Sugiyama, S. (1979) *Biochem. Biophys. Res. Commun.* **90**, 890–896

Onishi, S., Kawade, N., Itoh, S., Isobe, K. & Sugiyama, S. (1980*a*) *Biochem. J.* **190**, 527–532

Onishi, S., Isobe, K., Itoh, S., Kawade, N. & Sugiyama, S. (1980*b*) *Biochem. J.* **190**, 533–536

Onishi, S., Kawade, N., Itoh, S., Isobe, K., Sugiyama, S., Hashimoto, T. & Narita, H. (1981) *Biochem. J.* **198**, 107–112

Onishi, S., Itoh, S., Isobe, K., Togari, H., Kitoh, H. & Nishimura, Y. (1982) *Pediatrics* **69**, 273–276

Onishi, S., Miura, I., Isobe, K., Itoh, S., Ogino, T., Yokoyama, T. & Yamakawa, T. (1984*a*) *Biochem. J.* **218**, 667–676

Onishi, S., Ogino, T., Yokoyama, T., Isobe, K., Itoh, S., Yamakawa, T. & Hashimoto, T. (1984*b*) *Biochem. J.* **221**, 717–721

Ostrow, J. D. (1971) *J. Clin. Invest.* **50**, 707–718

Sargent, T. D., Yang, M. & Bonner, J. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 243–246

Sjödin, T., Hansson, R. & Sjöholm, I. (1977) *Biochim. Biophys. Acta* **494**, 61–75

Stoll, M. S., Zemone, E. A. & Ostrow, J. D. (1981) *J. Clin. Invest.* **68**, 134–141

von Dobeneck, H. (1979) in *The Porphyrins* (Dolphin, D., ed.), vol. 4, pp. 651–662, Academic Press, New York, San Francisco and London