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Kinetics of biliary excretion of the main two bilirubin photoproducts after injection into Gunn rats

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The kinetics of biliary excretion of the main two photoproducts after injection into Gunn rats were examined. The photoproducts that are obtained from experiments *in vitro* consist of unknown pigment, photobilirubin IXα and a small amount of (*ZZ*)-bilirubin IXα. It was confirmed previously that the first two photoproducts are identical with the main two photoproducts obtained *in vivo*. In experiments on four animals, the average of total biliary recoveries of unknown pigment was 81.4%, and that of photobilirubin IXα was 52.8%. The mean value of the half-lives for the appearance of photobilirubin IXα in the bile estimated by the Sigma-minus method was 29.8 min and that for unknown pigment was 4.3 min. The rate of thermal reversion of photobilirubin IXα to (*ZZ*)-bilirubin IXα in the bile at 37°C was very rapid, i.e. its half-life was 6.2 min.

The present view of the predominant pathway of bilirubin removal from the body in the course of phototherapy is geometric photoisomerization (McDonagh & Ramonas, 1978; McDonagh *et al.*, 1980; Onishi *et al.*, 1979, 1980*a*,*b*; Cohen & Ostrow, 1980). However, photochemical research on the *E*-configuration was carried out almost exclusively in unphysiological experimental conditions *in vitro* (McDonagh *et al.*, 1979; Lightner *et al.*, 1979*a*,*b*; Stoll *et al.*, 1979). A summary of our work on this problem reported previously (Onishi *et al.*, 1980*a*,*b*) is given below. Photobilirubin IXα in the serum of a hyperbilirubinaemic infant was demonstrated by h.p.l.c. to increase significantly during phototherapy. Corresponding to these changes in the serum, biliary unconjugated bilirubin concentration increased markedly but photobilirubin IXα was not detected in the bile. Large amounts of unknown pigment also appeared in the bile during phototherapy. Exact correspondence of retention time on h.p.l.c., diazo-reactivity, thermal and photochemical reversion and absorption-spectrum maxima was observed between the unknown pigment and photobilirubin IXα from biological fluids, and the comparable peaks 2 and 3 from experiments *in vitro*. However, little is known about the kinetics of bilirubin photoproducts in icteric infants undergoing phototherapy. It would be useful to explain the effect of light on bilirubin metabolism and the disposition of photoproducts by direct studies on the icteric rat. To elucidate the mechanism of these phenomena, we examined the kinetics of the biliary excretion of peaks 2 and 3 after their injection into Gunn rats.

Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

Materials and methods

Animals

Homozygous Gunn rats (*jj*) were obtained in 1964 from Dr. I. M. Arias (Albert Einstein College of Medicine, New York, NY, U.S.A.). After an initial crossbreeding with Wistar–Imamichi rats (*JJ*) the heterozygous offspring (*jJ*) was used to raise a pure line of Gunn rats. In adult animals the concentration of unconjugated bilirubin in serum was about 8–10 mg/100 ml. Four adult rats, weighing 190–340 g, were used for the present work.

Preparation of experimental animals and collection of bile

Under diethyl ether anaesthesia cannulas were placed into the common bile duct and a femoral vein. The animals were placed in restraining cages and kept in the dark under an i.r. heating lamp throughout the experiment. A synthetic solution

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containing glucose (2.6g/100ml), NaCl (70m-equiv./l) and sodium lactate (20m-equiv./litre) was infused into the femoral cannula at 3.0ml/h, except for the periods of injection of the solution of photoproducts. The solution (1ml) containing the photoproducts was injected via the femoral vein into the rat with an external bile fistula within a period of 1min. Bile was collected by the external bile fistula in Pyrex tubes shielded from light by aluminium foil as 15min fractions for a period of 4h. The volume of each bile specimen was measured. All bile samples were frozen immediately at −70°C and kept in the dark until analysed.

Kinetics of biliary excretion of photoproducts

The elimination and excretion of photoproducts was assumed to follow the one-compartment model proposed by Cummings *et al.* (1967). In that model, the plasma elimination rate constant for photoproducts is equal to the rate of appearance of photoproducts in the bile, which is assumed to be limited by the hepatic excretion of the photoproduct. According to the model, the half-lives of the photoproducts in the body are reflected by the half-lives for appearance of photoproducts in the bile. The kinetic constants for elimination of the photoproducts were determined solely from the biliary excretion rate by the Sigma-minus method described by Cummings *et al.* (1967). Semi-logarithmic plots of the percentage of the dose remaining to be excreted versus time were constructed for each animal.

Preparation of the solution of photoproducts

An equimolar solution (850μM) of human serum albumin and bilirubin in 0.02M-phosphate buffer, pH7.4, was irradiated under anaerobic conditions for 40min. One volume of the solution was vortex-mixed with 9vol. of chloroform for 30min and then centrifuged at 1000rev./min for 10min. The chloroform phase containing mainly (*ZZ*)-bilirubin IXα and the interphase of precipitated protein were discarded. The photoproducts in the supernatant aqueous phase were analysed by h.p.l.c. As shown in Fig. 1, the solution contained unknown pigment,

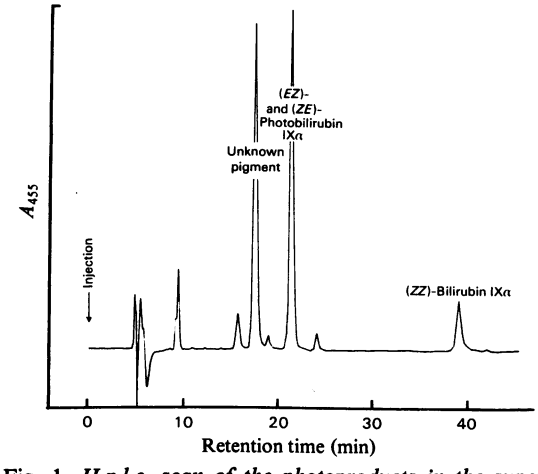

Fig. 1. *H.p.l.c. scan of the photoproducts in the supernatant aqueous phase*

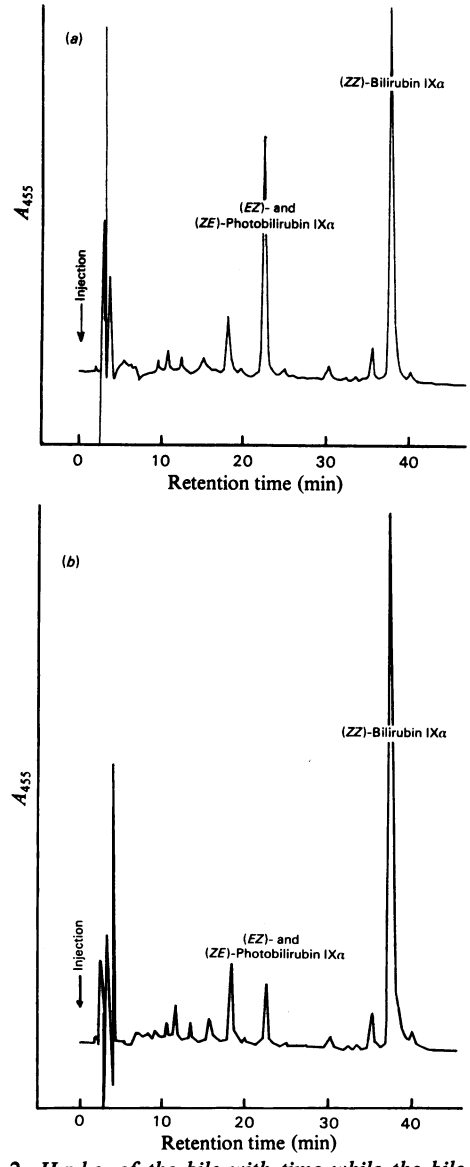

Fig. 2. *H.p.l.c. of the bile with time while the bile was incubated in the dark at 37°C*
The incubation was for 0min (*a*) and 14min (*b*).

photobilirubin IXα and a small amount of (*ZZ*)-bilirubin IXα. The quantity of photoproducts injected into each animal is shown in Table 1.

Chromatography, preparation of a calibration curve of peak area, sample preparation and the numbering of each peak separated by h.p.l.c. were carried out as described previously (Onishi *et al.*, 1980*a*,*b*).

Results and discussion

The decay of photobilirubin IXα in the bile

During collection of the bile, spontaneous reversion of photobilirubin IXα was inhibited by cooling to −70°C. H.p.l.c. analyses of the bile that was collected during 90–105 min after injection of photoproducts were performed in the course of incubation in the dark at 37°C. Figs. 2(*a*) and 2(*b*) show h.p.l.c. scans analysed at 0 and 14 min respectively. Photobilirubin IXα itself was clearly demonstrated only when the just-excreted bile was directly injected into the h.p.l.c. column without sample preparation. The half-life of photobilirubin IXα, i.e. the rate of thermal reversion to (*ZZ*)-bilirubin IXα in the bile at 37°C, was 6.2 min (Fig. 3). This value agrees approximately with the result of Stoll *et al.* (1979). Therefore, under ordinary experimental conditions, the amount of biliary excretion of photobilirubin IXα is equivalent to the total amount of photobilirubin IXα and (*ZZ*)-bilirubin IXα.

Kinetics of biliary excretion of photoproducts

During the control period, animals produced pale-yellow bile containing traces (0.095 mg/100 ml) of (*ZZ*)-bilirubin IXα at a steady state as shown in Fig. 4. On injection of the photoproducts, the bile turned abruptly yellow, which agrees with the observation of a rapid biliary response in the Gunn rat to light (Ostrow *et al.*, 1974; McDonagh & Ramonas, 1978). In experiments on four rats, the average of total recoveries of unknown pigment during the first 4 h was 81.4%, and that of photobilirubin IXα was 52.8%, as shown in Table 1. It is considered that the main reason for low biliary recovery of the photobilirubin IXα is due to the fact that its reversion to (*ZZ*)-bilirubin IXα occurs readily (Isobe & Onishi, 1981), in addition to its higher lipid solubility and therefore its higher affinity to albumin (Onishi *et al.*, 1980*a*), in comparison

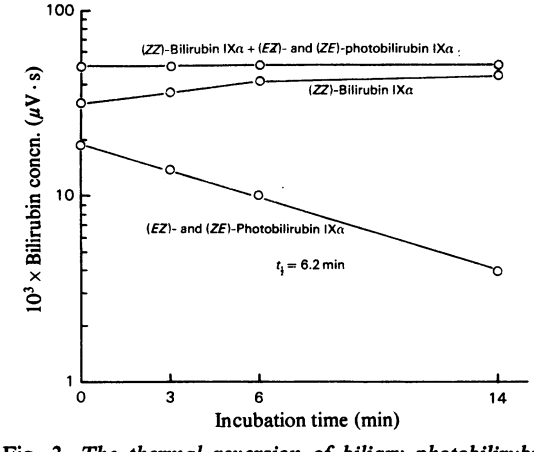

Fig. 3. *The thermal reversion of biliary photobilirubin IXa to (ZZ)-bilirubin IXa in the dark at 37°C*

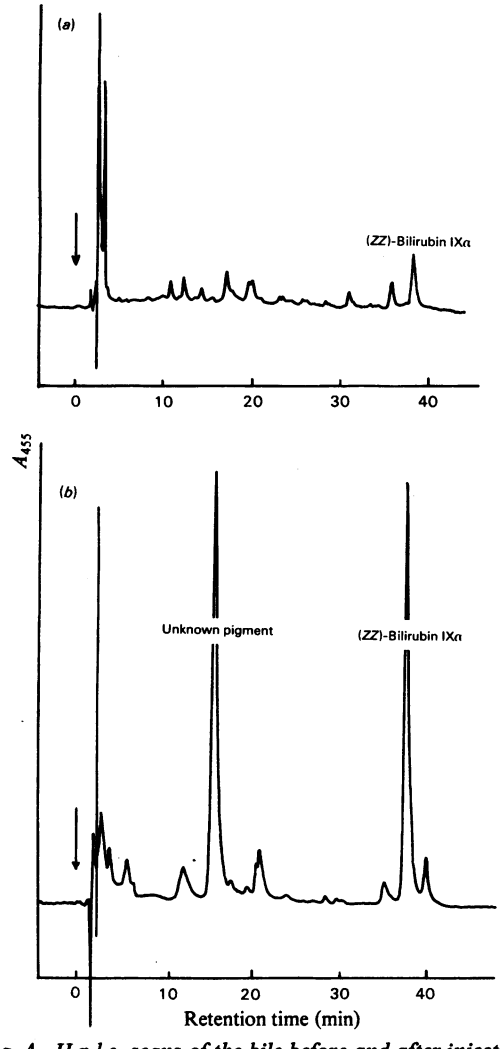

Fig. 4. *H.p.l.c. scans of the bile before and after injection of photoproducts*

The scan of the bile was during the control period (*a*) and 15–30 min after injection of photoproducts (*b*).

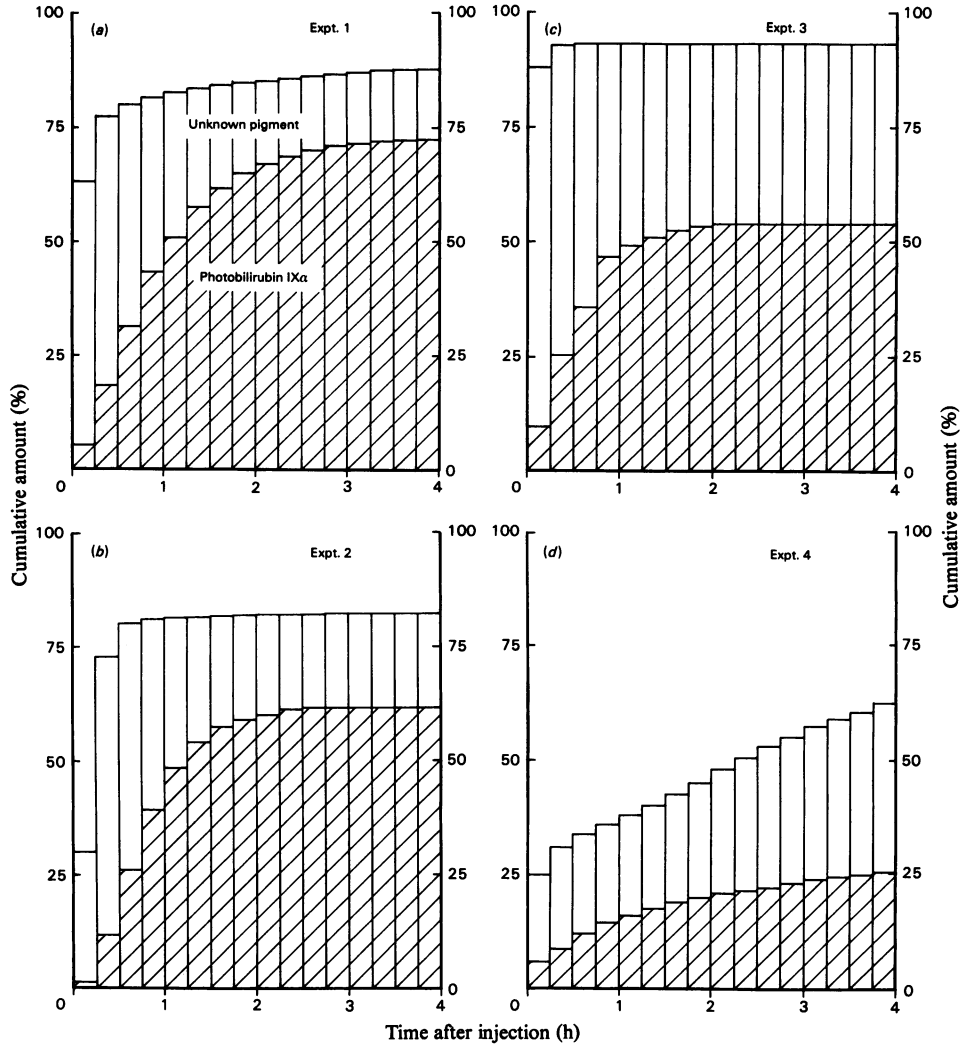

Fig. 5. *Cumulative biliary excretion of unknown pigment and photobilirubin IXα in the 4 h after intravenous injection of the photoproducts*

Cumulative amounts were expressed as percentages of the dose administered. The hatched columns are results for photobilirubin IXα and the empty columns are results for unknown pigment.

with those of unknown pigment. These values agree well with the result of experiments with labelled photoisomers administered to Gunn rats in the dark (Zenone *et al.*, 1977; Cohen & Ostrow, 1980). Figs. 5(*a*)–5(*d*) show cumulative biliary excretion of unknown pigment and photobilirubin IXα during 4 h after intravenous injection of photoproducts. Semilogarithmic plots in Figs. 6(*a*)–6(*d*) show the doses of unknown pigment and photobilirubin IXα to be excreted in the bile as a function of time by the Sigma-minus method in four Gunn rats that were intravenously given a dose of photoproducts. Half-lives for the appearance of the unknown pigment and photobilirubin IXα in the bile were calculated from data from the four animals. The mean value of half-lives for the appearance of the photobilirubin IXα in the bile was 29.8 min, and that of unknown pigment was 4.3 min, as shown in Table 1. This indicates that the overall elimination of unknown pigment from the body was far more rapid when compared with that of photobilirubin IXα. Therefore, the fact that photobilirubin IXα is detected only in the blood but not in the bile and, on the contrary, unknown pigment is observed only in the

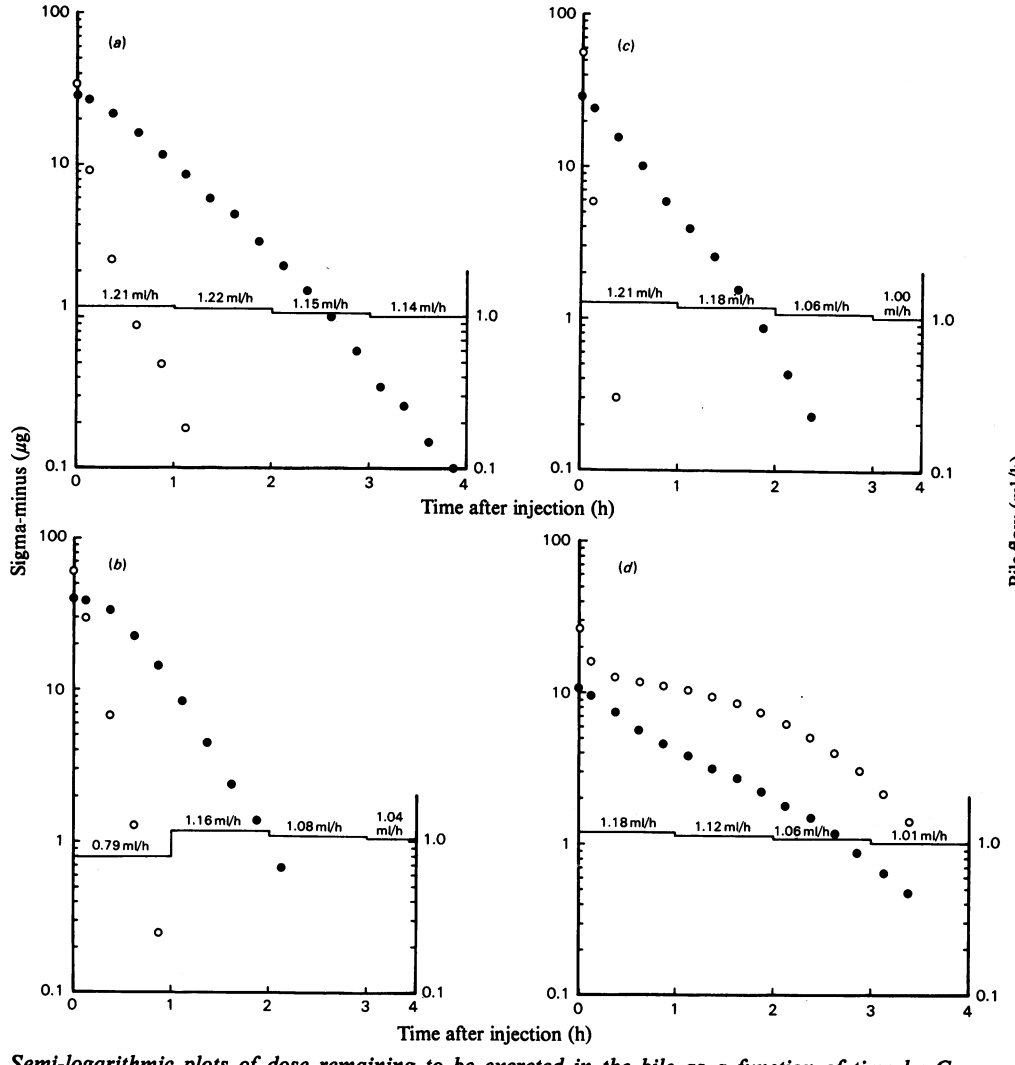

Fig. 6. *Semi-logarithmic plots of dose remaining to be excreted in the bile as a function of time by Gunn rats after intravenous injection of unknown pigment and photobilirubin IXα*
O, Unknown pigment; ●, photobilirubin IXα (*a*)–(*d*) represent different experiments.

Table 1. *Kinetics of biliary excretion of photobilirubin IXα and unknown pigment*
Results in parentheses are means ± S.D.

			Photobilirubin IXα			Unknown pigment		
Expt.	Body wt. (g)	Sex	Dose* (μg)	Recoveries† (%)	Half-life‡ (min)	Dose* (μg)	Recoveries† (%)	Half-life‡ (min)
1	320	♀	41.4	70.2	28	40.0	88.2	3.5
2	240	♀	66.2	60.8	20	75.2	81.9	6.0
3	190	♀	54.2	54.0	22	61.3	93.3	3.5
4	340	♀	42.8	26.3	49	41.4	62.1	—
			(51.3 ± 10.1)	(52.8 ± 16.4)	(29.8 ± 11.5)	(54.5 ± 14.6)	(81.4 ± 11.8)	(4.3 ± 1.2)

* Dose injected intravenously into Gunn rat.
† Biliary excretion of administered photoproducts.
‡ Half-lives for appearance in bile.

bile but not in the blood (Onishi *et al.*, 1979, 1980*a*,*b*) are supported by these experimental results.

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